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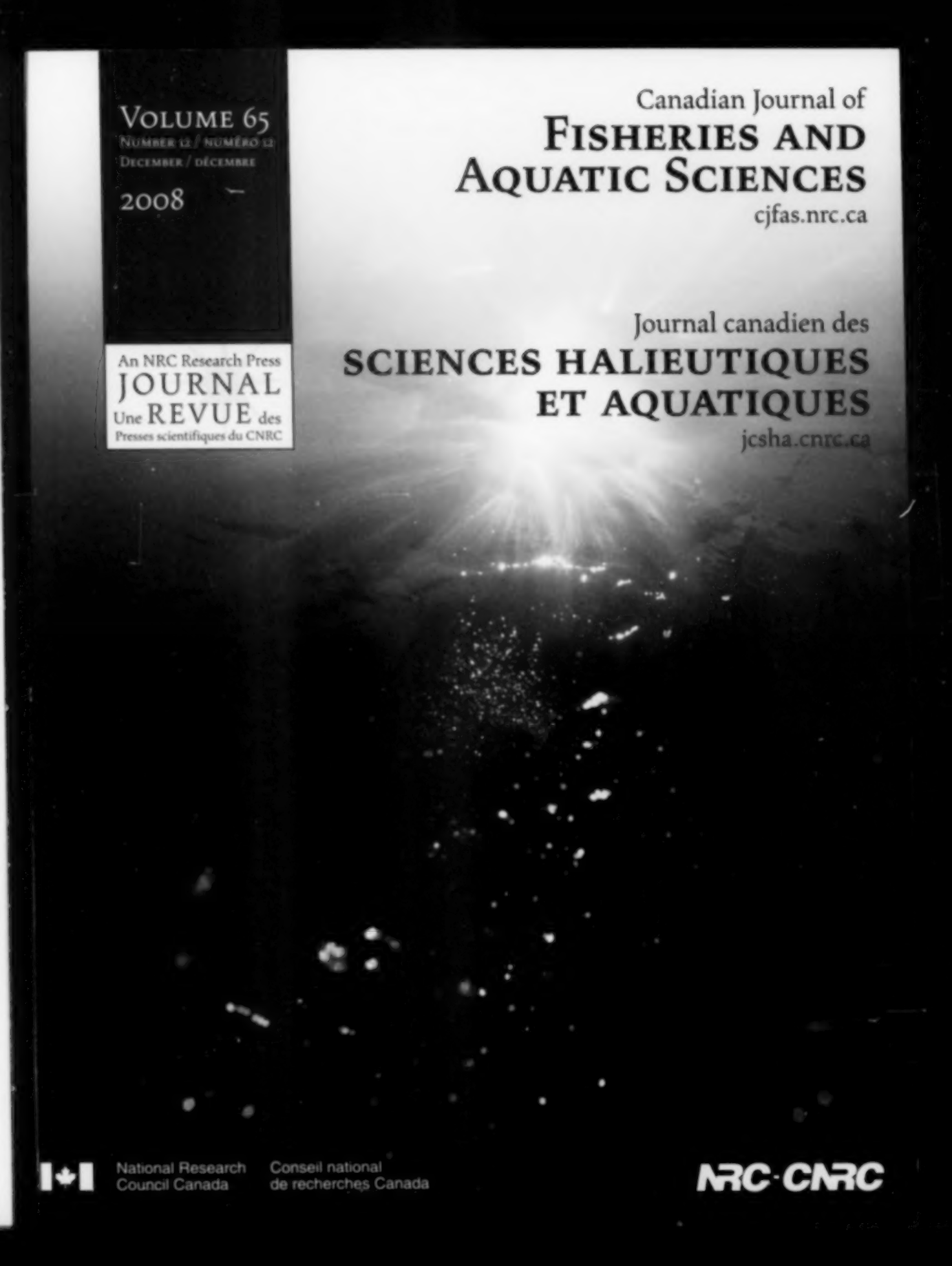

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Evaluating the knowledge base for expanding low-trophic-level fisheries in Atlantic Canada

Sean C. Anderson, Heike K. Lotze, and Nancy L. Shackell

Abstract: Over the last two decades, low-trophic-level fisheries have rapidly expanded in Atlantic Canada, largely compensating for collapsed groundfisheries; however, concerns have been raised regarding the limited background knowledge for many newly targeted species and their overexploitation in other regions. Using government stock assessments, we evaluated the amount of information available to assess population, fisheries, and ecosystem status in emerging (new since 1988), developing (expanding since 1988), and established fisheries on the Scotian Shelf. Emerging fisheries had significantly lower levels of population knowledge than developing and established fisheries. Importantly, knowledge was often lacking in basic population parameters such as growth rates, current biomass, and geographic range. In contrast, ecosystem knowledge, such as habitat disruption and recovery, was higher in emerging than established fisheries. Overall, quantitative knowledge was positively related to fishery value and greatest for 30- to 100-year-old fisheries. Although the number of government and general scientific publications greatly increased since 1990 for developing and established fisheries, publications for emerging fisheries remained at low levels. Emerging fisheries represent important socio-economic value in Atlantic Canada but may be progressing too rapidly for adequate knowledge to be gained, presenting a risk for their sustainable development.

Résumé : Au cours des deux dernières décennies, les pêches de niveau trophique inférieur ont connu une expansion rapide dans l'Atlantique canadien, compensant l'effondrement des pêches de fond; des inquiétudes ont, cependant, été formulées concernant le peu de connaissances de base disponibles sur ces nouvelles espèces ciblées et leur surexploitation dans d'autres régions. À l'aide d'évaluations de stocks gouvernementales, nous déterminons la quantité de données disponibles pour estimer le statut des populations, des pêches et des écosystèmes dans les pêches commerciales en émergence (nouvelles depuis 1988), en croissance (en expansion depuis 1988) et les pêches établies sur la plate-forme néo-écossaise. Il existe significativement moins de connaissances démographiques sur les pêches en émergence que sur les pêches croissantes ou établies. En particulier, il manque souvent de données sur les variables démographiques de base, telles que les taux de croissance, la biomasse courante et la répartition géographique. En revanche, les connaissances de l'écosystème, telles que les perturbations des habitats et leur récupération, sont plus grandes dans le cas des pêches en émergence que dans celui des pêches établies. Globalement, il existe une relation positive entre les informations quantitatives disponibles et la valeur de la pêche commerciale; ces informations sont le plus abondantes dans le cas de pêches en existence depuis 30 à 100 ans. Bien que le nombre de publications scientifiques gouvernementales et générales sur les pêches en croissance et les pêches établies ait augmenté depuis 1990, il reste bas dans le cas des pêches émergentes. Les pêches commerciales en émergence représentent une valeur socio-économique considérable dans l'Atlantique canadien, mais il se peut qu'elles progressent trop rapidement pour permettre l'acquisition de connaissances adéquates, ce qui représente un risque pour leur développement durable.

[Traduit par la Rédaction]

Introduction

The collapse of the groundfishery on the Canadian Scotian Shelf resulted in major ecosystem changes and economic challenges (Roy 1996; Worm and Myers 2003; Frank et al. 2005). This collapse was compensated for by the expansion of fisheries to lower trophic-level marine invertebrate and plant species (Lotze and Milewski 2004; Frank et al. 2005), thereby following a global trend of declining average trophic level of fisheries (Pauly et al. 1998, 2001). In Atlantic Canada, the monetary value of shrimp and crab landings alone now exceeds that of the former groundfishery (Frank et al. 2005); however, lessons from other parts of the world indicate that the rapid expansion and profit from low-trophic-level fisheries can be short term. For example, sea urchin and sea cucumber fisheries around the globe show rapid boom-and-bust patterns (Conand 2004; Berkes et al. 2006). This is not an entirely new phenomenon as can be seen in historical oyster fisheries (Kirby 2004). Recently, the sea cucumber fishery on the Canadian west coast was

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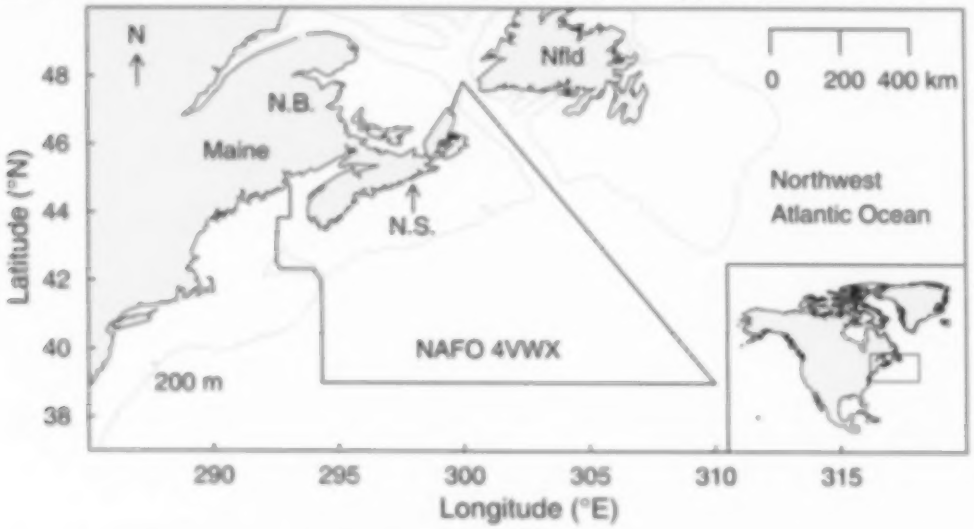
Fig. 1. Map of the study area showing NAFO (Northwest Atlantic Fisheries Organization) Divisions 4VWX and the Atlantic provinces of Canada (N.S. = Nova Scotia, N.B. = New Brunswick, Nfld = Newfoundland). The 200 m depth contour is shown.



on track to follow a similar pattern until stricter catch and license limitations and area closures were implemented (DFO 2002). It may be particularly important to ensure the long-term sustainable development of low-trophic-level fisheries in Atlantic Canada, since traditional groundfisheries are at low levels and so far not recovering (Frank et al. 2005).

In addition to their socio-economic importance, low-trophic-level species play important ecological roles in the marine ecosystem. Most low-trophic-level invertebrate and plant species act as prey to higher trophic levels, some provide vital three-dimensional habitat, and others provide filtering function and nutrient storage, regulating water quality on which other species depend (Rangeley 1994; DFO 1999; Dayton et al. 2002). Sea urchins in particular are known to be key engineers of the coastal algal community (Lawrence 1975). From an ecosystem perspective, a change in trophic balance or a change in the strength of species interactions increases the potential for instability (Frank et al. 2006, 2007). Soulé et al. (2005) have provided clear examples of altered ecosystems as a result of altering species interactions and argue that maintaining a trophic balance between strongly interacting species is necessary to ensure stability.

Despite their ecological and increasing economic importance, most efforts at collecting baseline fisheries data in Canada have been directed at higher trophic-level species (Rice and Rivard 2002). Concern has been raised about rapidly and simultaneously expanding low-trophic-level harvests while lacking sufficient baseline information to ensure sustainable fisheries development and marine ecosystem conservation (e.g., Rice and Rivard 2002; Lotze and Milewski 2004; Smith and Sainte-Marie 2004).

Perry et al. (1999) developed a framework for acquiring the necessary information to successfully manage a developing invertebrate fishery. Since these fisheries frequently occur for species with minimal existing knowledge, they propose three phases to acquiring population, fishery, and ecosystem knowledge: (Phase 0) existing information is compiled; (Phase 1) new information is obtained; and (Phase 2) management decisions are implemented, a commercial fishery begins, and monitoring to supplement knowledge continues. Further, the authors summarized a set of knowledge factors required to develop precautionary management strategies for emerging low-trophic-level fisheries (Perry et al. 1999).

In the present study, we used a modified version of the list provided by Perry et al. (1999) to evaluate the knowledge base for a set of emerging low-trophic-level fisheries on the Scotian Shelf. We tested whether the level of knowledge reported on population, fishery, and ecosystem parameters in government stock assessments and research documents varied between emerging and more established fisheries. We also analyzed factors that may explain differences in knowledge level and how knowledge changed over time. Based on our results, we discuss whether the fishing of emerging species is expanding more rapidly than knowledge can be gained and whether the available knowledge base is sufficient to enable sustainable management.

Methods

Selection of fisheries

We chose to investigate the knowledge base of established, developing, and emerging fisheries on the east coast of Canada (Fig. 1) because of the apparent shift from the historic predominance of groundfishing to rapidly expanding low-trophic-level harvesting after the groundfishery collapse. Our study focused on the Northwest Atlantic Fisheries Organization (NAFO) Divisions 4VWX on the Scotian Shelf and in the Bay of Fundy (Fig. 1).

To evaluate when a transition from groundfishing to lower trophic-level harvesting had occurred and to provide a cutoff for categorizing established, developing, and emerg-

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ing fisheries, we calculated the mean fished trophic level $\overline{TL}$ over each year k across all marine catch fisheries in NAFO Divisions 4VWX according to Pauly et al. (2001) as

$$(1) \qquad \overline{TL}_k = \sum_{i=1}^{m}(TL_i Y_{ik}) / \sum_{i=1}^{m}(Y_{ik})$$

where Y_i is the landings of species group i, TL_i is the trophic level of that species group, and m is the total number of species groups. We extracted catch statistics for all fisheries from 1960 to 2004 from the NAFO database (NAFO, Dartmouth, Nova Scotia, Canada). Data on trophic levels were obtained from the Sea Around Us Project (seaaroundus.org) and FishBase (fishbase.org). Based on the initiation of a relatively linear decline after at least 27 years of a steady mean fished trophic level, we chose 1988 as our cutoff.

To select a set of robust fisheries for our analysis, we first removed all small-scale sporadic fisheries with less than five consecutive data points. Second, we removed those fisheries that had not been substantially fished since the groundfishery collapse, which were all fisheries in which the 3-year running mean of catch did not exceed 100 t·year^{-1} after 1988. Third, we refined the scope of our study to those finfish fisheries that were similar to lower trophic-level fisheries in terms of habitat and fishing practices by removing all highly migratory, large pelagic, and diadromous fish species.

We crosschecked our list of NAFO fisheries against DFO (Fisheries and Oceans Canada) Annual Fisheries Statistics (L. Sonsini, Statistical Services, Fisheries and Oceans, 200 Kent St., Ottawa, Ontario, Canada, personal communication, 2005), which includes all economically valuable fisheries. We added any fisheries with catch of over 500 t·year^{-1} in 2004 (sea cucumber and rockweed), the most recent year for which we had fishery value statistics for all species. Rockweed landings data from 1962 to 1985 were obtained from Canadian Atlantic Fisheries Scientific Advisory Committee (1991). Rockweed and sea cucumber landings from 1986 to 2004 were obtained from DFO Annual Fisheries Statistics (L. Sonsini, personal communication, 2005).

We aimed to divide the fisheries into those that were established prior to the groundfishery collapse (established), those that existed before but were expanded with the collapse (developing), and those that started as new fisheries after the collapse (emerging). We therefore used the following rules to select the three fisheries groups: (*i*) A fishery was considered emerging if the catch did not pass 500 t·year^{-1} or 10% of the long-term (1960–2004) maximum for more than 2 consecutive years prior to 1988, and the fishery had been fished for at least 5 consecutive years after 1988. If a fishery was not classified as emerging, it was considered (*ii*) developing if the mean catch after 1988 was 25% or greater than in the 1960–1988 period and (*iii*) established if the mean catch had declined or was less than 25% greater after 1988.

Given the length of the available documents on many established fisheries, we limited our knowledge analysis to a representative sample ($n = 6$) of two groundfish species (Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*)), one pelagic species (Atlantic herring (*Melanogrammus aeglefinus*)), two flatfish species (yellowtail flounder (*Limanda ferruginea*) and Atlantic halibut (*Hippoglossus hippoglossus*)), and one predominantly deepwater species (Atlantic redfishes (*Sebastes* spp.)) (Table 1). Among the developing and emerging species, several fisheries were difficult to evaluate. While the Canadian catches for spiny dogfish (*Squalus acanthias*) resembled our emerging fisheries, there was substantial catch in NAFO Divisions 2–6 by Russia and the United States since the early 1960s (DFO 2007*b*). We therefore categorized this species as developing. Catch statistics for sandgaper (*Mya arenaria*) and silver hake (*Merluccius bilinearis*) revealed substantial discrepancies between NAFO and DFO statistics and were therefore excluded. Finally, there were a number of emerging fisheries with no published DFO reports so far, including red hake (*Urophycis chuss*), roundnose grenadier (*Coryphaenoides rupestris*), Atlantic hagfish (*Myxine glutinosa*), northern quahog (*Mercenaria mercenaria*), and sculpins (family Cottidae). These fisheries may form an important part of the overall picture; however, we could not evaluate their knowledge level according to our method. In the end, our sample for the knowledge evaluation included seven developing and eight emerging fisheries (Table 1).

Knowledge analysis

Based on Perry et al. (1999), we developed a list of 21 population, 12 fisheries, and 7 ecosystem knowledge parameters that would aid in developing a precautionary management plan for emerging low-trophic-level fisheries (Appendix A, Table A1). The original table was designed to aid in developing management for an individual fishery (Perry et al. 1999). To provide an objective comparison for a set of fisheries, we refined the knowledge factors so that each factor could be designated as absent, present in a qualitative sense, or present in a quantitative sense. If a knowledge factor was deemed irrelevant to a certain fishery, such as size at maturity for rockweed (Appendix A, Table A1), it was removed from the evaluation of that fishery. Four of the factors could only be evaluated as qualitative knowledge and were excluded from the quantitative evaluation (Appendix A, Table A1).

To evaluate the knowledge level for each fishery, we searched for the most recent DFO Stock Status Reports (SSR), Canadian Stock Assessment Secretariat (CSAS) Science Advisory Reports (SAR, replaced SSR in 2005), DFO Habitat Status Reports (HSR), and CSAS Research Documents (Table 1). We restricted our evaluation of individual knowledge factors to these documents for three reasons: (*i*) we expected that individuals involved in the management of these fisheries would refer to these documents as their primary source of information; (*ii*) it allowed a consistent approach in the comparison of all fisheries studied; and (*iii*) these documents represented the most recent information considered in each fishery's assessment and management. Where available, we used the most recent of each type of document that evaluated the stock (SSR, SAR, HSR, and Research Documents). Where multiple fishing areas were evaluated in the same year (for example, Jonah crab), we included all fishing areas together. Therefore, if a knowledge factor was demonstrated in one of the documents, it was reported present for that species.

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Table 1. Fisheries investigated, regulatory measures, and data sources for knowledge analysis.

Common name	Scientific name	Regulatory measure	Data source
Established fisheries			
Atlantic cod	*Gadus morhua*	Quota	Clark and Perley 2006; DFO 2006*a*
Haddock	*Melanogrammus aeglefinus*	Quota	Mohn and Simon 2004; DFO 2006*b*
Atlantic halibut	*Hippoglossus hippoglossus*	Quota	Zwanenburg et al. 2003; DFO 2006*c*
Atlantic redfishes	*Sebastes* spp.	Quota	McClintock 2006; DFO 2004*a*
Yellowtail flounder	*Limanda ferruginea*	Quota	Stone and Legault 2003*a*, 2003*b*; DFO 2003
Atlantic herring	*Clupea harengus*	Quota	Power et al. 2007*a*, 2007*b*; DFO 2007*a*
Developing fisheries			
Spiny dogfish	*Squalus acanthias*	Quota	DFO 2007*b*
American lobster	*Homarus americanus*	Effort	Robichaud and Pezzack 2007; DFO 2007*c*
Snow crab	*Chionoecetes opilio*	Quota	Choi and Zisserson 2007; DFO 2004*b*, 2007*d*, 2007*e*
Northern shrimp	*Pandalus borealis*	Quota	Covey and King 2006*a*, 2006*b*; DFO 2007*f*
Sea scallop	*Placopecten magellanicus*	Quota	Smith et al. 2007; DFO 2007*g*, 2007*h*, 2007*i*
Periwinkle	*Littorina littorea*	None	DFO 1998*a*
Rockweed	*Ascophyllum nodosum*	N.B. quota	DFO 1998*b*
		N.S. area-based	DFO 1999
Emerging fisheries			
Red crab	*Chaceon quinquedens*	Effort, quota	DFO 1997
Atlantic rock crab	*Cancer irroratus*	Effort	Robichaud et al. 2000*b*; Tremblay and Reeves 2000; DFO 2000*a*, 2000*b*
Jonah crab	*Cancer borealis*	Effort	Adams et al. 2000; Robichaud et al. 2000*a*, 2000*b*; DFO 2000*c*, 2000*d*, 2000*e*
Ocean quahog	*Arctica islandica*	Quota	Roddick et al. 2007*b*, 2007*c*; DFO 2007*j*
Arctic surfclam	*Mactromeris polynyma*	Quota	Roddick et al. 2007*a*; DFO 2007*j*
Atlantic surfclam	*Spisula solidissima*	Quota	DFO 1998*a*
Sea urchin	*Strongylocentrotus droebachiensis*	N.B. quota, N.S. ecosystem-based	Miller and Nolan 2000; DFO 2000*f*, 2000*g*
Sea cucumber	*Cucumaria frondosa*	—	DFO 1996*b*

For each fishery, we recorded whether qualitative or quantitative knowledge was reported for each knowledge factor. Qualitative knowledge was defined as any qualitative information provided for the knowledge factor in an appropriate context. Quantitative knowledge was defined as a numeric value, range of values, or, in the case of geographically based factors, a map. Except for those knowledge factors in the fishery category, quantitative knowledge factors had to be based on fisheries-independent research, surveys, or models because of the potential bias in data obtained from fisheries catch (for example, size–age structure based only on catch composition). We recorded these instances separately, but treated them as qualitative knowledge in our analyses. If quantitative knowledge was present, we assumed the same for qualitative knowledge. We maintained a separation of qualitative and quantitative knowledge to avoid making an assumption about the relative importance of each.

As a first step to compare the knowledge level across fisheries, we calculated the percentage of reported qualitative and quantitative factors within each knowledge group (population, fishery, and ecosystem) for each fishery. We then calculated the mean percentage and 95% confidence intervals (CI) of reported knowledge within each group of fisheries (established, developing, and emerging).

To assess the similarity among fisheries within and between the groups when the knowledge of each factor was taken into account, we performed Kruskal's nonmetric multidimensional scaling (MDS) (Kruskal and Wish 1978) using the isoMDS function in the MASS library (Venables and Ripley 2002) for the statistics package R (R Development Core Team 2008) on the qualitative and quantitative results using a binary calculated distance matrix.

We used an analysis of odds ratios to compare individual knowledge factors among established, developing, and emerging fisheries. Odds ratios provide a comparison statistic and a measure of confidence when comparing two groups with presence–absence data (Cooper and Hedges 1994). Further, they can be combined across studies or factors to provide a pooled comparison estimate. We used a Mantel–Haenszel common odds ratio to draw inference about whether the odds of finding each type of knowledge (population, fishery, ecosystem) were greater or lower in emerging fisheries compared with developing or established fisheries.

Odds ratios are calculated from binary presence–absence data. Given the following cell frequencies for a single knowledge factor:

	Present	Absent
Group_1	A	B
Group_2	C	D

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the odds ratio o comparing Group$_2$ with Group$_1$ for a given factor i can be written as

$$(2) \qquad o_i = \frac{A/B}{C/D} = \frac{AD}{BC}$$

The standard error of the log odds ratio for each factor SE(log(o_i)) can be calculated

$$(3) \qquad \mathrm{SE}(\log(o_i)) = \sqrt{\frac{1}{A} + \frac{1}{B} + \frac{1}{C} + \frac{1}{D}}$$

and used to calculate the 95% CI for each factor

$$(4) \qquad 95\%\ \mathrm{CI}\ \log(o_i) = \log(o_i) \pm \mathrm{SE}(\log(o_i))$$

In many scenarios, including cases with small sample sizes but many factors, the Mantel–Haenszel technique provides a method of estimating a fixed-effect common odds ratio $\bar{o}_{\mathrm{MH}}$ across factors, given that homogeneity Q (calculation described in Cooper and Hedges (1994)) among the factors is not rejected (Cooper and Hedges 1994). The common odds ratio can be calculated as

$$(5) \qquad \bar{o}_{\mathrm{MH}} = \frac{\sum_{i=1}^{k} A_i D_i / T_i}{\sum_{i=1}^{k} B_i C_i / T_i}$$

where i represents each factor, k is the number of factors being compared, and T_i is the total number of observations for each factor across both groups ($T_i = A_i + B_i + C_i + D_i$). The calculation of a CI for $\bar{o}_{\mathrm{MH}}$ is described in Cooper and Hedges (1994).

Traditionally, when one of the cells (A, B, C, or D) is zero, 0.5 is added to each of the cells for that factor to prevent an odds ratio of zero or undefined (Cooper and Hedges 1994). The addition of 0.5 is done assuming that given a large enough sample size, we would not find complete presence or absence of knowledge for any factor across a fishery group (established, developing, and emerging); however, when combining odds ratios into a pooled estimate, this correction is unnecessary and may introduce bias in small sample sizes (Cooper and Hedges 1994; Bradburn et al. 2007). For ease of visual interpretation, we have shown the odds ratios for each factor with the 0.5 correction where appropriate, but shown and reported the Mantel–Haenszel common odds ratios using the original data. The results of the common odds ratio analysis should be interpreted with the knowledge that not all factors are independent (e.g., gear type and gear selectivity).

Temporal trends and possible drivers

For many of the emerging and some of the developing fisheries, there were only one or two CSAS documents available, so we could not evaluate a temporal increase in knowledge over time. Instead, we used an evaluation of the number of CSAS documents (SSR, SAR, HSR, or Research Documents) published each year for each species since 1977, when an online database of publications began (www.dfo-mpo.gc.ca/csas/csas/Publications/Pub_Index_e.htm). Similarly, to assess how general scientific knowledge of these species may have developed over time, we queried the Web of Science database (Thomson Scientific, isiknowledge.com) for each of our species. We limited our search to articles of any language in the "Science Citation Index Expanded" database. We searched for each species by scientific name in the topic or title and in which Canada was listed in the address field. Prior to 1970, no publications matched our queries. For both the CSAS documents and Web of Science publications, we calculated a mean number of publications per 5-year span for each fishery group. Standard error bars were calculated assuming a negative binomial distribution of the count data. To approximate the increase in indexed publications in the Web of Science over time, we obtained the total number of publications indexed with Canada in the address field for each year.

Lastly, we evaluated possible explanatory drivers for the difference in knowledge levels observed among fisheries. Therefore, we analyzed whether qualitative and quantitative knowledge level was a function of the longevity of a fishery or the value of the catch. We hypothesized that reported knowledge might be a function of the age of a fishery or that it might be correlated with the fisheries' value, as reported in the year that many of the emerging fishery reports were last written (2000). We tested these two hypotheses using least squares regression. We calculated the percentage of reported qualitative and quantitative knowledge for each fishery. Longevity data (the year that each fishery began as a directed fishery) were obtained from the CSAS reports used in the knowledge analysis (Table 1). Atlantic herring and haddock dates were obtained from Perley (1852), sea scallop from Lotze and Milewski (2004), yellowtail flounder from Stone et al. (2004), and Atlantic surfclam from the NAFO landings. Fisheries value data for 2000 were extracted from DFO Annual Fisheries Statistics (L. Sonsini, personal communication, 2005).

Results

On the Scotian Shelf, a strong shift in fishing occurred during 1980–2000 when established groundfisheries experienced strong declines while developing and emerging low-trophic-level fisheries rapidly increased (Figs. 2*a*, 3). This transition was marked by the decline of the mean fished trophic level that began around 1988 and continued throughout the 1990s and early 2000s (Fig. 2*b*). Overall, catches of developing species increased 5-fold over a 30-year period from the 1965–1975 average to 2000, while catches of emerging species increased 10-fold in just 10 years from 1988 to 1998 (Fig. 2*a*). Established fisheries declined 2-fold since 1988. Although this gap is shrinking, they still present a 1- to 2-fold higher catch than developing fisheries and a 5- to 10-fold higher catch than emerging fisheries (Fig. 2*a*). From 1988 to 2000, the value of the investigated emerging fisheries increased on average 13-fold and that of developing fisheries 2.5-fold, while the value of established fisheries halved (Table 2); however, many of the emerging and developing fisheries, except lobster, rockweed, and sea cucumbers, have experienced a decline in landings in recent years (Fig. 3).

The output of CSAS reports was most consistent for the established fisheries and some developing fisheries, includ-

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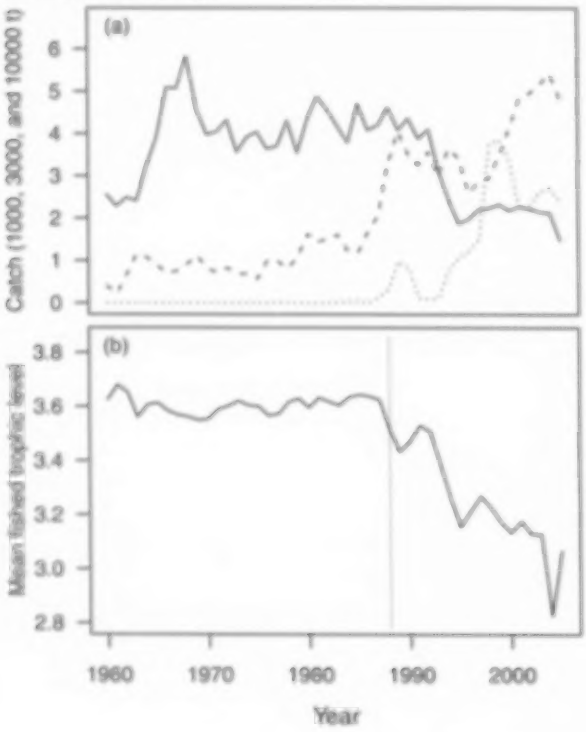
Fig. 2. (*a*) Mean annual catch of the investigated fisheries divided into established (solid line, 10 000 t increments), developing (expanded since 1988, dashed line, 3000 t increments), and emerging (new since 1988, dotted line, 1000 t increments) categories. (*b*) Mean fished trophic level for NAFO Divisions 4VWX. The vertical line represents the chosen cutoff year (1988) for distinguishing fisheries groups.



ing lobster, snow crab, shrimp, and scallop (Fig. 3, circles). The output was less consistent for developing dogfish, periwinkle, and rockweed as well as all emerging fisheries. Five emerging fisheries had no CSAS document at all and were thus not included in our analysis (see Methods). Of the investigated eight emerging fisheries, three have only one published report, and all but two (red crab, rock crab, Jonah crab, Atlantic surfclam, sea urchins, and sea cucumbers) have not had a CSAS document published since 2000 (Fig. 3).

The level of reported knowledge among the three fisheries groups (established, developing, and emerging) depended on the knowledge factors considered. Quantitative population knowledge was more than two times lower in emerging fisheries compared with established or developing fisheries (Figs. 4*a*, 4*d*, 4*g*). Five of the eight emerging fisheries demonstrated less than 20% of the possible quantitative population knowledge factors, whereas all the developing and established fisheries had at least this much (Figs. 4*a*, 4*d*, 4*g*). Fishery knowledge was most consistently reported across the three groups, with the exception of quantitative fisheries knowledge in the Atlantic surfclam and sea cucumber fisheries (Figs. 4*b*, 4*e*, 4*h*). In contrast, ecosystem knowledge was higher for developing and emerging fisheries than for established fisheries (Figs. 4*c*, 4*f*, 4*i*). There was never more than one of the investigated ecosystem parameters discussed in the established fisheries documents, and quantitative ecosystem knowledge was never reported (Fig. 4*c*, Appendix A, Table A1). Besides the consistently low ecosystem knowledge of established fisheries, there was the least variability of reported fisheries knowledge in the developing fisheries (Fig. 4*e*).

Nonmetric MDS across all knowledge factors presented a better approximation of the similarity of the fisheries groups across quantitative knowledge factors (Fig. 5, stress = 13.3%) than qualitative factors (S.C. Anderson, unpublished data, stress = 19.1%). For both qualitative and quantitative knowledge, developing fisheries (with the exception of rockweed and periwinkle) were the most closely grouped, indicating similar patterns of knowledge across knowledge factors. Established fisheries were the next most closely grouped, showing considerable overlap with developing fisheries. In contrast, the emerging fisheries were the most scattered. The pattern of reported knowledge of sea cucumber and Atlantic surfclam was the most dissimilar to the other fisheries (Fig. 5).

The level of knowledge reported for individual knowledge parameters varied considerably across parameters and fisheries groups, as well as between qualitative and quantitative knowledge reported (Appendix A, Table A1). Importantly, emerging fisheries had limited knowledge on spatial structure (e.g., metapopulation, degree of aggregation, seasonal migration) and basic quantitative population parameters such as growth, recruitment, and natural mortality rates as well as current biomass levels and geographic range; however, knowledge on virgin biomass level was higher in emerging (reported in two fisheries) than in developing or established fisheries (Appendix A, Table A1).

In our odds ratio meta-analysis, homogeneity across the knowledge parameters was not rejected within each of the comparisons allowing for the evaluation of a common odds ratio (Table 3). The odds of finding population knowledge were significantly greater in the developing and established fisheries than the emerging fisheries. The odds were approximately six times greater for quantitative factors and seven times greater for qualitative factors that we would find population knowledge in developing fisheries over emerging fisheries (Fig. 6*a*, Table 3). In the established–emerging fisheries comparison, the odds were approximately four times greater for finding quantitative or qualitative population knowledge in the reports (Fig. 6*b*, Table 3). Within the population parameters, the odds of finding quantitative knowledge on growth rate, fecundity at size, and lifespan were significantly higher in the developing fisheries than in emerging fisheries (Fig. 6*a*). The odds of finding quantitative knowledge on geographic range and growth rate were significantly greater in established fisheries than in emerging fisheries (Fig. 6*b*). Although not significant at $P = 0.05$, our results suggest that the odds of finding knowledge of current biomass may be greater in developing and established fisheries compared with emerging fisheries (Fig. 6*a*, 6*b*).

The overall odds of finding fishery knowledge parameters reported in the emerging fisheries were not significantly different from the developing or established fisheries, although they were nearly so where comparing quantitative knowl-

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Fig. 3. Individual catch trends for established (solid line), developing (dashed line), and emerging (dotted line) fisheries in NAFO Divisions 4VWX. The vertical line represents the cutoff year (1988), and the open circles identify years with Canadian Stock Assessment Secretariat (CSAS) documents published for the particular fisheries. Spiny dogfish (*Squalus acanthias*) landings represent Canadian catches only, but substantial USA and Russian landings have been recorded for the same population throughout NAFO Divisions 2–6 since the early 1960s.

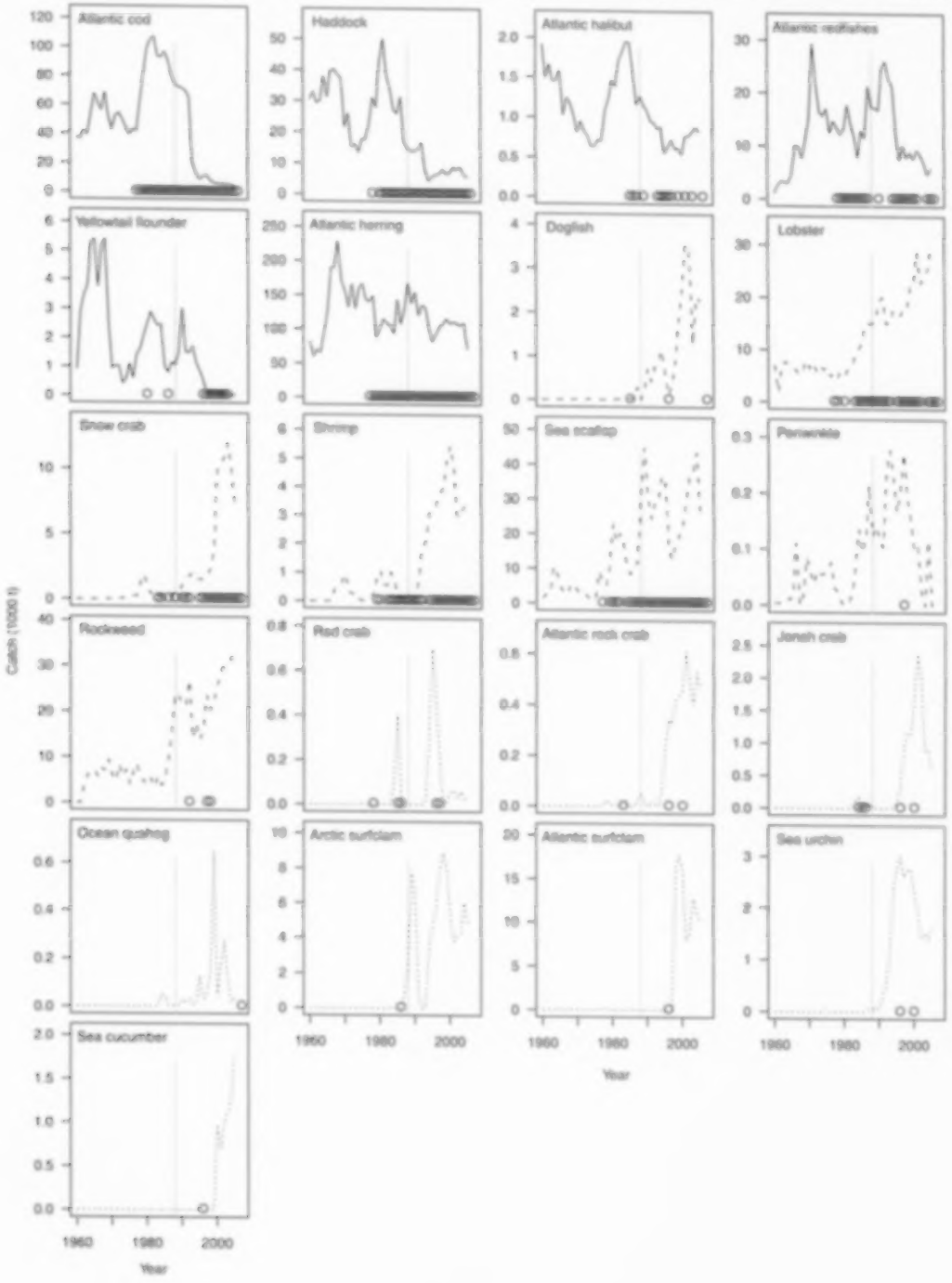

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Table 2. Annual reported fishery catch value in 1988 and 2000 for established, developing, and emerging fisheries (data modified from L. Sonsini, personal communication, 2005).

	Value (CAN$)		
Fishery	1988	2000	% change
Established			
Atlantic cod	45 398 416	11 506 701	25.4
Haddock	17 071 125	13 912 413	81.5
Atlantic halibut	6 011 185	4 641 570	77.2
Atlantic redfishes	5 240 115	4 764 210	90.9
Yellowtail flounder	838 114	79 262	9.5
Atlantic herring	26 666 019	15 653 575	58.7
Mean	16 870 829	8 426 289	50.0
Developing			
Spiny dogfish	296	854 852	288 801.4
American lobster	122 791 881	291 481 992	237.4
Snow crab	1 609 349	52 453 976	3 259.3
Northern shrimp	146 091	11 913 709	8 155.0
American sea scallop	33 404 456	33 982 342	101.7
Periwinkle	122 241	172 322	141.0
Rockweed	945 445	1 023 510	108.3
Mean	22 717 108	55 983 243	246.4
Emerging			
Red crab	0	42 115	—
Atlantic rock crab	18 614	606 665	3 259.2
Jonah crab	3 196	2 932 321	91 749.7
Ocean quahog	2 912	138 498	4 756.1
Arctic surfclam	2 723 598	18 311 901	672.3
Atlantic surfclam	0	10 559	—
Sea urchin	35 811	5 954 098	16 626.5
Sea cucumber	0	181 749	
Mean	348 016	3 522 238	1 012.1

Note: There was a 33.7% change in the Canadian dollar between 1988 and 2000: CAN$1.00 in 1988 = CAN$1.34 in 2000 (Statistics Canada, Consumer Price Indexes for Canada, 1914–2006 (V41690973 series); www.bankofcanada.ca/en/rates/inflation_calc.html).

edge of emerging and developing fisheries (Figs. 6*c*, 6*d*, Table 3). Further, there may be greater odds of having maps of specific fishery locations (by catch or effort) in developing compared with emerging fisheries.

Ecosystem knowledge, especially quantitative knowledge, was the least known for all fisheries groups but higher for developing and emerging fisheries than for established fisheries (Fig. 4; Appendix A Table A1). The odds of finding ecosystem parameters reported in emerging fisheries were not significantly different from developing fisheries (Fig. 6*e*), but the odds of finding qualitative ecosystem parameters in the emerging fisheries were significantly greater than in the established fisheries (Fig. 6*f*).

Our analysis of the temporal trends in available knowledge revealed that there were large increases in the publication rate for developing and established fisheries in CSAS documents since the 1980s and in Web of Science documents since the 1970s (Fig. 7). In contrast, there was only a small increase in CSAS and Web of Science publications for emerging fisheries. In the period from 2000 to 2004, the average number of CSAS documents published per fishery was about five times greater for developing and seven times greater for established fisheries compared with emerging fisheries (Fig. 7*a*). These differences in publication rates among the three fisheries groups were similar in the Web of Science with an average of about four times more publications for developing fisheries and 10 times more publications for established fisheries compared with emerging fisheries (Fig. 7*b*). In comparison with the total number of Canadian publications in the Web of Science, which linearly increased over time, the publications on developing and established fisheries strongly increased in the 1990s (Fig. 7*b*).

Fisheries that began approximately 30–100 years ago and high-value fisheries generally had the most reported knowledge (Fig. 8). A quadratic regression relating overall quantitative knowledge to the log of fishery age (Fig. 8*a*) and a linear regression relating quantitative knowledge to the log of fishery value (Fig. 8*b*) were significant. Reported qualitative knowledge was weakly related to the log of fishery age (quadratic regression, $r = 0.39$, $P = 0.233$) and fishery value in 2000 (linear regression, $r = 0.43$, $P = 0.053$).

Discussion

We evaluated whether emerging low-trophic-level fisheries on the Scotian Shelf had similar levels of knowledge

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Fig. 4. Percentage of reported qualitative (open circles) and quantitative (solid circles) knowledge levels for established (*a*, *b*, *c*), developing (*d*, *e*, *f*), and emerging (*g*, *h*, *i*) fisheries. Diamonds represent mean and 95% confidence intervals for percentage of qualitative (open) and quantitative (closed) knowledge reported for each fisheries group.

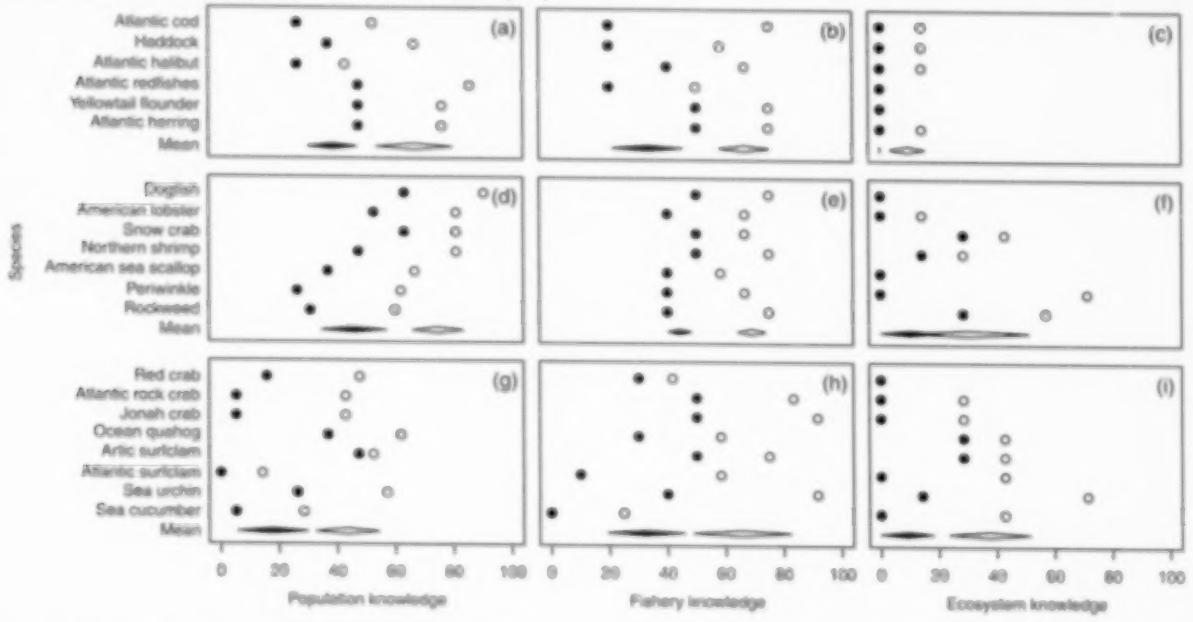
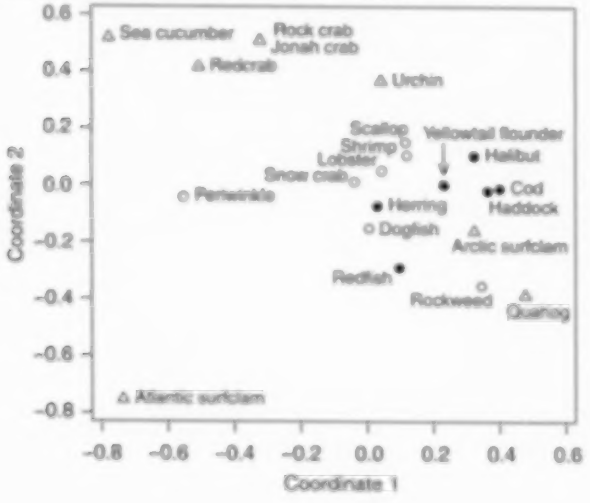


Fig. 5. Kruskal's nonmetric multidimensional scaling (MDS) for quantitative knowledge, based on all individual knowledge factors using a binary calculated distance matrix (stress = 11.3%). Solid circles represent established fisheries, open circles show developing fisheries, and open triangles indicate emerging fisheries.



reported for population, fishery, and ecosystem parameters compared with developing and established fisheries. Based on the most recently published government stock assessments and research documents for each species, we found that emerging fisheries had significantly lower levels of population knowledge reported than developing and established fisheries, but higher levels of ecosystem knowledge than established fisheries. The lack of important quantitative population parameters, such as biomass, growth rates, and geographic range, may hinder any thorough population assessment and impair a precautionary approach to management (Perry et al. 1999). The slightly higher ecosystem knowledge, on the other hand, may indicate that we have started to incorporate information on the ecological role of target species and the ecosystem effects of the fishery into management. Overall, however, emerging fisheries appear to have been developed more rapidly in terms of catches and value than knowledge has been acquired. Management based on the limited existing and only slowly increasing knowledge may be inadequate to ensure the long-term sustainability of emerging fisheries, which are of high ecological and increasing socio-economic importance in Atlantic Canada and elsewhere.

Status of emerging fisheries

Over the past two decades, a rapid expansion of low-trophic-level fisheries occurred in Atlantic Canada following the depletion and collapse of major groundfisheries (Lotze and Milewski 2004; Frank et al. 2005). On the Scotian Shelf, landings of several already existing invertebrate and plant fisheries, such as shrimp, lobster, and rockweed, increased greatly during the 1980s and 1990s. Simultaneously, a set of mainly invertebrate fisheries, such as rock crab, surfclams, sea urchins, and sea cucumbers, emerged that were previously not commercially targeted. In recent years, however, many emerging and developing fisheries experienced declining landings. The reasons for this may be manifold. Some catch trends may be declining because of lack of a market, some may be due to more restrictive quotas (e.g., DFO 2007*f*), while others may be due to de-

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Table 3. Test for heterogeneity Q of the effect size and Mantel–Haenszel common odds ratio meta-analysis, comparing the qualitative and quantitative knowledge base of emerging with developing and established fisheries across three knowledge categories.

Comparison	df	Q[a]	P_Q	$\hat{o}_{MH}$[b]	Z[c]	P_Z
(*i*) Emerging–developing, qualitative						
Population	20	13.01	0.877	7.50	5.95	<0.001
Fishery	11	9.20	0.604	1.22	0.55	0.580
Ecosystem	6	7.48	0.278	0.72	–0.77	0.444
(*ii*) Emerging–developing, quantitative						
Population	18	13.37	0.769	6.64	5.35	<0.001
Fishery	9	4.96	0.838	2.35	1.87	0.061
Ecosystem	6	3.14	0.791	1.16	0.22	0.823
(*iii*) Emerging–established, qualititative						
Population	20	24.07	0.239	3.46	4.28	<0.001
Fishery	11	13.89	0.239	1.06	0.16	0.872
Ecosystem	6	7.74	0.258	0.04	–2.78	0.006
(*iv*) Emerging–established, quantitative						
Population	18	21.82	0.240	3.64	3.86	<0.001
Fishery	9	6.20	0.720	1.06	0.13	0.895
Ecosystem[d]	6	—	—	—	—	—

[a]The assumption of homogeneity of the effect sizes was not violated in any of the comparisons (P_Q statistically nonsignificant).

[b]The Mantel–Haenszel common odds ratio $\hat{o}_{MH}$ tests how much more likely we are to find knowledge in the second group. A value of 1 indicates that the odds are equal.

[c]The Z statistic tests whether $\hat{o}_{MH}$ is significantly different from 1.

[d]Established fishery quantitative knowledge was not reported, making a common odds ratio using our methodology undefined.

clines in catch per unit effort possibly indicating changes in abundance (e.g., DFO 2000*g*). The lack of recent stock assessments for many species makes such a distinction difficult. Such rapid increases and declines are not unusual for low-trophic-level fisheries, as can be seen, for example, in sea urchin and sea cucumber fisheries around the globe (Conand 2004; Berkes et al. 2006). The expansion of fisheries to lower trophic levels in the food chain was reflected by a strong decline in average trophic level of the catch since the groundfish depletion, suggesting the "fishing down the food web" phenomenon described by Pauly et al. (1998, 2001). This expansion may have several underlying drivers. (*i*) The collapse of the groundfishery created pressure to search for other socio-economic opportunities to earn an income and find employment (Roy 1996). (*ii*) Global markets for a number of invertebrate species were developed. For example, Berkes et al. (2006) have suggested the development of sea urchin fisheries worldwide may be driven by patterns of "roving banditry" in which the sequential decline of other sea urchin fisheries coupled with Asian demand and globalized trade may drive the development of new fisheries. (*iii*) Only those species that occur in sufficient abundance can be regarded as viable fisheries options. The depletion of higher trophic-level predators such as Atlantic cod has likely released many prey species from strong predation pressure (Worm and Myers 2003; Frank et al. 2005), indirectly enhancing their abundance. Increasing low-trophic-level landings in Atlantic Canada were accompanied by a strong increase in the value of developing and emerging species. Many of the emerging fisheries had exceeded $1 000 000·year^{-1} by 2000. For example, the sea urchin fishery increased 166-fold in value from 1988 to 2000, exceeding $5 000 000·year^{-1}, but experienced declining catches in recent years. Similarly, one of the newest emerging fisheries on the Scotian Shelf, sea cucumber, increased in value from zero in 1997 to nearly $400 000·year^{-1} in 2005 (L. Sonsini, personal communication, 2005) a period in which catches increased from zero to 1800 t·year^{-1}. On the Canadian west coast, the sea cucumber fishery experienced a similarly rapid increase in landings and value over a span of 5–7 years before catch per unit effort declined substantially and restrictive management allowed the stock to recover (DFO 2002). Experience elsewhere with both sea urchin and sea cucumber fisheries suggests the potential for rapid boom-and-bust patterns in low-trophic-level fisheries (Andrew et al. 2002; Conand 2004; Berkes et al. 2006).

Knowledge base

There were clear differences in knowledge levels reported for emerging, developing, and established fisheries. Importantly, five emerging fisheries on the Scotian Shelf had no CSAS documents published at all as of 2008 and could thus not be evaluated in our analysis. Six of the remaining eight had not been evaluated since at least 2000. We included established fisheries as a reference of what a realistic target level of knowledge might be. Admittedly, catches for these fisheries have declined in recent decades, and several have closed because of overfishing, which suggests that having

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Fig. 6. Meta-analysis of odds ratios for reported knowledge factors in emerging versus developing (*a*, *c*, *e*) and established (*b*, *d*, *f*) fisheries. Odds ratios and 95% confidence intervals are shown for qualitative (open circles, broken lines) and quantitative (solid circles, solid lines) knowledge of each knowledge factor. The fixed-effect Mantel–Haenszel overall odds ratios and 95% confidence intervals are shown for qualitative (open diamonds) and quantitative (solid diamonds) knowledge. All data are plotted on a log scale. Individual factors are shown with 0.5 added to all comparisons with a zero, but common odds were calculated with original values.

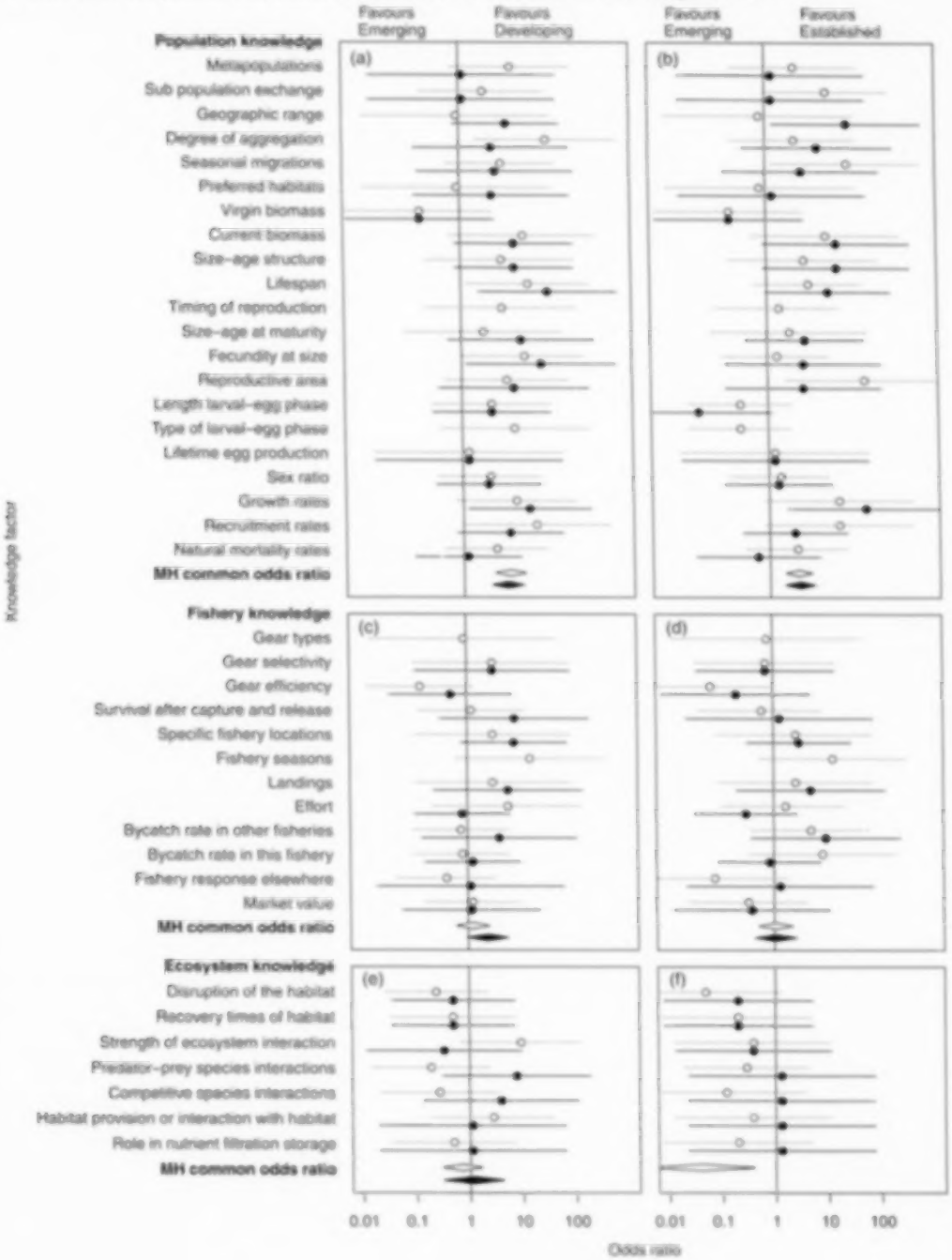

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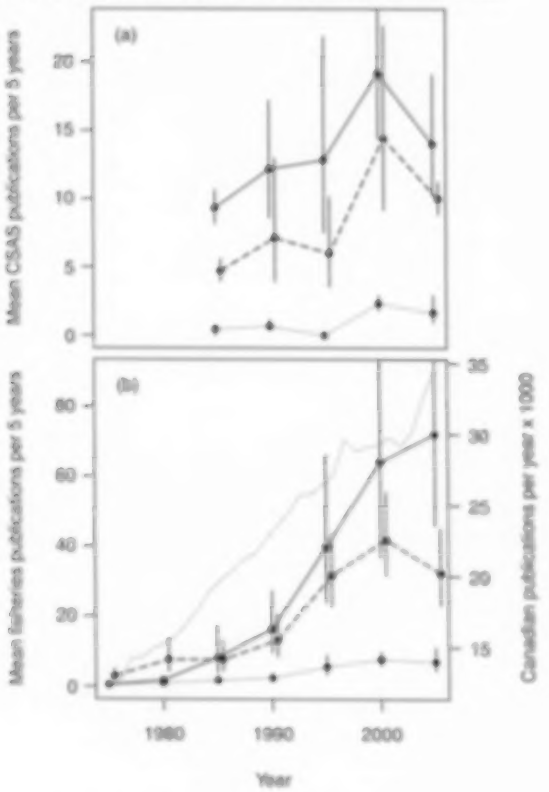
Fig. 7. Mean number of (*a*) all Canadian Science Advisory Secretariat (CSAS) Research Documents, Stock Status Reports, Science Advisory Reports, and Habitat Status Reports and (*b*) articles indexed in the Web of Science "Science Citation Database" per 5-year span for established (solid line), developing (dashed line), and emerging (dotted line) fisheries. Year values indicate first year of span (e.g., 2000–2004). Standard error bars are shown based on a negative binomial distribution of the count data. The grey line in (*b*) represents the total number of articles per year indexed by the Web of Science in which an author's address was in Canada.



knowledge does not necessarily ensure sustainability. Still, having knowledge is likely a prerequisite for developing a sustainable fishery.

The largest gap between emerging and more established fisheries was in population knowledge. In many emerging fisheries, some of the most basic quantitative population parameters such as growth rates, current biomass, and geographic range were lacking, which may impair proper population assessment. While obtaining fisheries-independent data on population parameters is cost- and labour-intensive, these data are vital to proper population assessment and fisheries management (Hilborn and Walters 1992; Chen et al. 2003). With a stronger population knowledge base comes lower uncertainty in stock assessment (Chen et al. 2003) and an increased likelihood that long-term ecologically and socially beneficial decisions can stand up against short-term political and financial pressures.

Three factors may have contributed to an underestimation of the population knowledge gap for some emerging and developing fisheries. (*i*) Five emerging fisheries could not be evaluated because a CSAS report had not yet been published. (*ii*) Many invertebrate species exhibit a greater diversity of life-history characteristics than finfish and may require a wider range of population parameters to be known for proper management (Caddy 2004). (*iii*) The amount of general scientific knowledge, especially for emerging but also for developing fisheries, is much reduced compared with established fisheries, and thus less general knowledge is available to be incorporated into government stock assessments.

An essential reference point that was not reported for almost every species, virgin biomass, was known for some emerging fisheries. With these fisheries, we have the possibility of collecting data from the beginning of the fishery; however, these "virgin biomass" levels may not be natural but the result of predator or competitor release in severely altered ecosystems (Worm and Myers 2003; Frank et al. 2006; Myers et al. 2007). For more established fisheries, virgin biomass levels could be estimated using analyses of carrying capacity or historical data, but this has been attempted for only a few species (Myers et al. 2001; Lotze and Milewski 2004; Rosenberg et al. 2005).

Overall, fishery-related knowledge levels were similar across the types of fisheries. The availability of fisheries-dependent data is not surprising, since the fisheries directly acquire them and they are a focus of fisheries stock assessments; however, we were surprised to find that in some emerging fisheries, the most basic quantitative information on landings, effort, and fishing location were not known. Also, many of the assessment and research reports did not include knowledge from similar fisheries in other regions in the world, such as the population response to fishing. Such knowledge could be of particular use to emerging fisheries, such as the sea urchin and sea cucumber fisheries, for which valuable experience exists elsewhere. It was also our observation that landings and particularly effort data for individual fisheries and management areas are currently widely spread among DFO divisions, often split into short time series, and difficult to access and compile. As suggested earlier by Pauly et al. (2001), a consistent, long-term, and easily accessible series of Canadian landings and effort data would greatly aid in the analysis and management of fisheries resources.

Ecosystem knowledge was variable across fisheries but lowest in established fisheries. Management of some emerging and developing fisheries has made extensive use of ecosystem factors, while many other fisheries appear to be managed using a more traditional single-species model. For example, in the Nova Scotian sea urchin fishery, harvesters must demonstrate the maintenance or enhancement of habitat carrying capacity in exchange for exclusive access to fishing zones (DFO 2000*f*). The southwestern New Brunswick sea urchin fishery, in contrast, has used a more traditional total allowable catch (TAC) management strategy despite lacking sufficient fishing effort and population data (DFO 2000*g*).

Since 1996, under the Oceans Act (Department of Justice 1996), DFO has been given a mandate to gather information relating to the habitats and ecosystems that support all fisheries and base its management on this knowledge. The low

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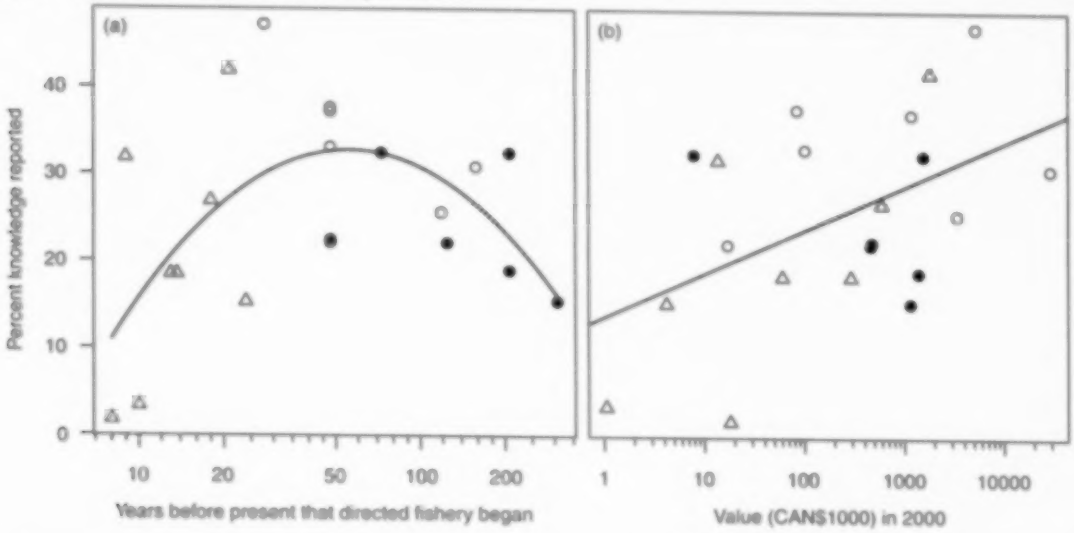
Fig. 8. Relationship between percentage of quantitative knowledge reported for established (solid circles), developing (open circles), and emerging (open triangles) fisheries with (*a*) the longevity of a fishery (i.e., the number of years a directed fishery has existed) ($r = 0.60$, $P = 0.019$) and (*b*) the value of a fishery (CAN$) in the year 2000 ($r = 0.50$, $P = 0.021$). Lines indicate (*a*) quadratic (quadratic coefficient $\gamma = -5.76$, $P = 0.008$) and (*b*) linear regression fits.



levels of reported ecosystem knowledge may indicate that the Oceans Act has not been implemented in most fisheries. For example, the collapse and lack of recovery of Atlantic cod on the Scotian Shelf may be attributed to not only overfishing but also changes to the ecosystem structure as a consequence of high exploitation rates (Frank et al. 2006, 2007). In the case of lower trophic-level species, exploitation of forage fish, invertebrates, and plants affects the availability of prey, vital three-dimensional habitat, and water quality to the entire species community (Dayton et al. 2002; Lotze et al. 2006). Thus, ecosystem knowledge on these species is important for their fisheries and overall ecosystem management. To ensure ecosystem conservation while maintaining sustainable fisheries, ecosystem knowledge factors should be acquired and incorporated into an ecosystem-based management (EBM) approach (Pikitch et al. 2004).

Based on our results, we argue that a balanced knowledge base that includes basic population, fisheries, and ecosystem knowledge factors may be the best initial strategy to account for uncertainties as the fishery develops; however, not all knowledge factors may be equally important in developing sustainable management strategies. Perry et al. (1999) recommended an iterative process between knowledge requirements and proposed management approaches, such that not all types of knowledge would be required depending on the approaches selected. Also, a weighting scheme could be developed to account for the relative importance among individual knowledge factors. We did not incorporate these approaches into our analysis because both are likely to differ among the various species and management approaches, making it difficult to compare across fisheries.

Among the fisheries we evaluated, the increasing number of government and general scientific publications over the past two decades on developing and established fishery species may reflect an increase in knowledge over time. Publications on emerging fishery species, however, remained at low levels despite their rapid expansion. This led us to question whether fishing of emerging species may develop faster than knowledge is or can be acquired. For many established fisheries, resource inaccessibility, effort limitations (seasons, gear, vessels) and export restrictions slowed their development while scientific knowledge and management expertise developed over centuries (Lotze 2004). Technological progress and globalization has released emerging fisheries from these constraints, and market demand as well as globalized trade can spur rapid increases in exploitation (Berkes et al. 2006). Recent examples of rapidly increased and sequentially depleted emerging fisheries around the globe (Andrew et al. 2002; Uthicke and Conand 2005; Berkes et al. 2006) may indicate the great pressure on emerging fisheries and the lack of knowledge for proper management.

In our analyses, fisheries that began 30–100 years ago had a stronger knowledge base than more recent fisheries, which could be partly attributed to their greater value, but also to the timing of their development: before the collapse of the groundfisheries. Thus, there may have been a head start in gathering data before the pressure to enhance exploitation increased. In contrast, emerging fisheries were developed after the groundfishery collapse with strong pressure to expand quickly and little time to gather information; however, some of these emerging fisheries are now 10–15 years old and still lack adequate population knowledge. The lower knowledge reported in older fisheries (>100 years) could be partly attributed to the lower ecosystem knowledge reported in these fisheries.

We also found the overall knowledge level to be correlated to the total value of the fishery. Thus, only fisheries that reach a certain economic importance may be adequately evaluated and monitored by management agencies. This

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means that we miss out on collecting important baseline information on populations and the ecosystem before exploratory fisheries are expanded to higher levels of commercial fishing. Collecting baseline population information before large-scale harvesting is allowed is critical for a number of reasons. First, when funding and commitment to such management directives are not allocated from the beginning, momentum is difficult to regain later once fishing has been established (Guenette et al. 1998). Second, if populations are substantially altered before sufficient population knowledge can be gained, we will have lost vital information about their natural state and contributed to a shifting baseline syndrome (Pauly 1995).

Potential solutions

It is likely unreasonable to argue that all basic population and ecosystem knowledge should be acquired before a fishery is opened; however, to successfully manage developing and emerging fisheries, especially for species with minimal existing knowledge, a certain level of research may be necessary before the fishery begins. Perry et al. (1999) developed a framework for acquiring the necessary population, fishery, and ecosystem knowledge based on three phases (outlined in Introduction). If followed, this framework could ensure that basic knowledge parameters are acquired before and throughout the development of a new fishery.

Establishing refuges at the beginning of a fishery during Phase 0 (Perry et al. 1999) would provide information on an unfished proportion of the population that could be monitored over time, establishing important reference points of unfished parts of populations and ecosystems. None of the investigated emerging fisheries have incorporated designated marine reserves into management in which no fishing of any kind is permitted. Given our results, the relative immobility of several invertebrate and plant species, the necessity to collect information on unfished populations, and the costs and time required to gather that information, we suggest that fishing reserves would work well as part of a management strategy for emerging fisheries.

Another integral component of developing sustainable fisheries could be EBM (Pauly et al. 2002), particularly when combined with an overall management plan that incorporates quotas, effort restrictions, and gear control (Stefansson and Rosenberg 2005). Such an approach would likely incorporate the following into management: trophic interactions, evaluations of the impact of various gears on habitat, and marine reserves of suitable location and substantial size (Pauly et al. 2002). Of the emerging fisheries investigated, only the Nova Scotian sea urchin fishery has trophic interactions explicitly incorporated into management. Given the destructive impacts of bottom trawl fishing gear on marine habitat and biodiversity (e.g., Messieh et al. 1991; Hiddink et al. 2006; Tillin et al. 2006), the use of such gear in parts of the New Brunswick sea urchin fishery and the Scotian Shelf sea cucumber fishery is incongruent with EBM and the recognized importance of seafloor habitats (Department of Justice 1996).

The present study draws attention to the lack of population knowledge in emerging fisheries and the lack of ecosystem knowledge in established fisheries in Atlantic Canada. We suggest that monitoring of population and ecosystem parameters for existing emerging and established fisheries, respectively, should gain highest priority, followed by insurance measures such as area closures, precautionary quotas, effort controls, and gear restrictions where appropriate (Stefansson and Rosenberg 2005). Given their ecological roles, socio-economic value, and recent patterns of expansion and depletion elsewhere, the identified knowledge gaps may represent a risk to the sustainable development of marine resources.

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Appendix A

Table A1 appears on the following pages.

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Table A1. Reported knowledge (1, qualitative; 2, quantitative; —, not reported) for established, developing, and emerging fisheries in Atlantic Canada.

	Fishery status																			
	Established						Developing							Emerging						
Knowledge factor*	cod	had	hal	rdf	fl	her	dg	lob	snc	shp	sca	per	rcw	rec	roc	jc	osj	arc	atc	su	sc
Population parameters																					
Metapopulations	—	—	—	1	—	1	1	1	—	1	1	—	—	—	—	—	1	—	—	—	—
Subpopulation exchange	—	1	—	1	1	1	1	—	—	1	—	—	—	—	—	—	1	—	—	—	—
Geographic range	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1	1	2	2	1	1	1†
Degree of aggregation	—	—	—	2	—	2	1	1	1	1	1	2	—	—	—	—	—	—	—	1	—
Seasonal migrations	1	1	1	1	2	1	2	1	1	—	—	1	NR	—	1†	1	—	—	—	—	0
Preferred habitats	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1
Virgin biomass	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	2	—	—	—
Current biomass	2	2	2	2	2	2	2	2	2	2	2	1	2	1†	—	—	2	2	—	2	—
Size–age structure	2	2	2	2	2	2	2	2	2	2	2	1	2	1†	1†	1†	2	2	—	2	—
Lifespan	—	2	—	2	2	2	2	—	2	2	2	2	2	—	—	—	1	2	—	—	—
Timing of reproduction‡	1	1	—	1	1	1	1	1	1	1	1	1	1	1	1	1	—	—	1	1	1
Size–age at maturity	2	2	1	2	2	2	2	2	2	2	2	2	NR	2	1†	1†	2	2	—	2	1
Fecundity at size	—	1	—	2	—	—	2	2	2	1	—	2	NR	—	1†	—	—	1	—	—	—
Reproductive area	1	1	1	1	1	2	2	1	2	—	—	—	NR	1	—	—	—	—	—	—	—
Length larval–egg phase	—	—	—	1	1	—	2	2	2	2	2	2	—	2	2	2	—	—	—	2	2
Type of larval–egg phase‡	—	—	—	1	1	—	1	1	1	1	1	1	1	1	1	1	—	—	—	1	1
Lifetime egg production	—	—	—	—	—	—	—	—	—	—	—	—	NR	—	—	—	—	—	—	—	—
Sex ratio	—	—	—	2	1†	2	2	2	2	1†	—	—	NR	2	—	1†	2	—	—	—	—
Growth rates	2	2	2	2	2	2	2	2	2	2	1	—	2	—	—	—	1	2	—	1	—
Recruitment rates	1	2	2	1	2	1	2	2	2	2	2	1	1	—	—	—	1	2	—	2	—
Natural mortality rates	1	1	—	—	2	1	1	2	1	2	1	—	—	—	—	—	2	2	—	1	—
Fishery parameters																					
Gear types‡	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Gear selectivity	2	2	2	—	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—
Gear efficiency	—	—	—	—	1	—	—	1	—	—	—	—	2	—	1	1	2	2	1	1	—
Survival after capture and release	1	—	—	—	—	—	2	—	—	—	—	—	2	—	—	1	—	—	—	1	—
Specific fishery locations	1	1	2	1	2	2	2	2	2	2	1	2	1	1	2	2	1	1	1	1	—
Fishery seasons‡	1	1	1	1	1	1	1	1	1	1	1	1	1	—	1	1	—	1	—	1	—
Landings	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	—
Effort	1	1	1	—	2	2	1	2	2	2	2	1	1	2	2	2	—	2	1	2	—
Bycatch rate in other fisheries	1	—	1	2	2	1	2	—	1	—	—	1	—	—	1	1	—	—	1	—	1
Bycatch rate in this fishery	1	1	2	1	1	2	—	—	2	2	2	—	1	—	2	2	1†	2	—	1	—
Fishery response elsewhere	—	—	—	—	—	—	1	—	—	1	—	—	—	—	—	—	1	1	—	1	1
Market value	—	—	—	—	—	1	—	1	—	1	—	2	—	—	1	1	—	—	—	2	—
Ecosystem parameters																					
Disruption of the habitat	—	—	—	—	—	—	—	—	—	—	—	1	2	—	1	1	2	2	—	1	—
Recovery times of habitat	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	2	2	—	—	—
Strength of ecosystem interaction	—	—	—	—	—	—	—	—	1	1	—	1	1	—	—	—	—	—	—	2	—

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Table A1 (*concluded*).

	Fishery status																					
	Established						Developing							Emerging								
Knowledge factor[a]	cod	had	hal	rdf	fl	her	dg	lob	snc	shp	sea	per	rcw	rex	ros	jc	esq	arc	atic	su	sc
Predator–prey species interactions	1	1	1	—	—	1	—	1	2	2	—	1	—	—	1	1	1	1	1	1	1
Competitive species interactions	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	1	1	1
Habitat provision or interaction	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	—	1	—
Role in nutrient filtration storage	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1	—	1

Note: Cod, Atlantic cod; had, haddock; hal, Atlantic halibut; rdf, Atlantic redfishes; fl, yellowtail flounder; her, Atlantic herring; dg, spiny dogfish; lob, American lobster; snc, snowcrab; shp, northern shrimp; sea, American sea scallop; per, periwinkle; rcw, rockweed; rex, red crab; ros, Atlantic rock crab; jc, Jonah crab; esq, ocean quahog; arc, Arctic surfclam; atic, Atlantic surfclam; su, sea urchin; sc, sea cucumber; NR, not relevant to that fishery.

[a]Knowledge parameters modified from Perry et al. (1999).

[b]Quantitative population knowledge was not fisheries-independent, or fisheries knowledge was inferred from sources outside the fishery (bycatch rate estimated from research trawls). These instances were treated as qualitative knowledge in our analyses.

[c]These parameters were only evaluated for qualitative knowledge.

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Perry, R.I., Walters, C.J., and Boutillier, J. 1999. A framework for providing scientific advice for the management of new and developing invertebrate fisheries. Rev. Fish Biol. Fish. **9**: 125–150. doi:10.1023/A:1008946522213.

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Establishing highly accurate production-age data using the tree-ring technique of crossdating: a case study for Pacific geoduck (*Panopea abrupta*)

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Abstract: We apply the tree-ring technique of crossdating to generate highly accurate age data and evaluate error in annual growth increment (annual growth zone) counts for long-lived Pacific geoduck (*Panopea abrupta*) in the Tree Nob Islands, northern British Columbia, Canada. Crossdating is the most fundamental procedure of tree-ring analysis and is based on the tendency of environmental variability to synchronize the growth of all individuals at a given site. By cross-matching these synchronous growth "bar codes", all growth increments can be correctly identified and assigned the correct calendar year, including the innermost year of recruitment. In this analysis, a total of 432 geoduck individuals were aged using crossdating methods as well as annual growth increment counts. The entire crossdating process was completed using visual techniques, requiring no additional equipment beyond a microscope or microprojector. When compared with crossdated ages, growth increment counts consistently underaged Pacific geoduck, particularly in the oldest individuals. These inaccuracies obscured major recruitment pulses and underestimated the rarity of strong recruitment events. To date, crossdating has been used to develop growth chronologies in a variety of marine and freshwater bivalve and fish species, but no study has demonstrated how the technique can be used to dramatically and economically improve accuracy in age data.

Résumé : Nous utilisons une technique d'interdatation servant à l'étude des anneaux de croissance des arbres pour produire des données très précises de détermination de l'âge et pour évaluer l'erreur du dénombrement des incréments annuels de croissance (zone de croissance annuelle) chez la panope du Pacifique (*Panopea abrupta*), un mollusque à forte longévité, aux îles Tree Nob, nord de la Colombie-Britannique, Canada. L'interdatation est la technique la plus fondamentale de l'analyse des anneaux des arbres et elle se base sur la tendance qu'a la variabilité environnementale à synchroniser la croissance de tous les individus à un site donné. Par l'appariement croisé de ces « codes à barres » de croissance synchronisée, il est possible d'identifier correctement tous les incréments de croissance et d'assigner la bonne année du calendrier, même à l'année la plus ancienne de recrutement. Dans notre analyse, nous avons déterminé l'âge de 432 panopes à l'aide de méthodes d'interdatation et de dénombrement des incréments annuels de croissance. Tout le processus d'interdatation a pu être complété à l'aide de techniques visuelles, ne nécessitant aucun équipement autre qu'un microscope ou qu'un microprojecteur. Par rapport à la détermination de l'âge par interdatation, le dénombrement des incréments de croissance sous-estime systématiquement l'âge des panopes du Pacifique, particulièrement chez les individus plus âgés. Ces imprécisions masquent les principales périodes de recrutement plus intenses et sous-estiment la rareté des événements de fort recrutement. À ce jour, l'interdatation a servi à élaborer des chronologies de croissance chez une variété d'espèces de bivalves et de poissons marins et d'eau douce, mais aucune étude n'avait démontré comment la technique peut être utilisée pour améliorer de façon spectaculaire et économique la précision des données de détermination de l'âge.

[Traduit par la Rédaction]

Introduction

In fisheries management, accurate estimates of fish age are required to establish population growth and mortality rates, recruitment patterns, and the effects of human activities and natural environmental variability on fish populations. Consequently, hundreds of thousands of fish and shellfish ages are estimated each year, typically by counting annual growth increments (i.e., annual growth zones or annuli) in scales, otoliths, shells, or other hard structures (Campana and Thorrold 2001; Valero et al. 2004). These age estimates often involve widely agreed upon criteria for interpreting annual growth increments, as well as any blind areas, checks, or other irregular patterns. Yet they may still contain considerable error, especially for longer-lived species (Beamish and McFarlane 1983; Boehlert and Yoklavich 1984).

To address this issue, a number of validation techniques have been developed to assess the accuracy of age estimates (Campana 2001). In one approach, fish or shellfish are physically or chemically tagged, released, and recaptured at a later date to verify the periodicity of growth increment formation over the time elapsed (Beamish and Chilton 1982; Heifetz et al. 1998). More specific to long-lived species, the absolute age of material in the core of calcified structures

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can be determined using the decay of naturally occurring radioisotopes (Turekian et al. 1975; Shaul and Goodwin 1982; Turekian et al. 1982) or the sudden pulse of marine ^{14}C that followed nuclear testing in the 1950s and 1960s (Kalish 1993; Campana 1997; Ebert and Souton 2003). Any disagreements between age estimates from annual growth increment counts and radiometric techniques would indicate biases in ageing criteria. A number of other age validation techniques are also available for fish or shellfish, each with its own set of advantages, limitations, and ranges in accuracy and precision (Campana 2001).

Borrowing from tree-ring science (dendrochronology), crossdating offers an additional validation tool that could provide high levels of ageing accuracy, particularly for long-lived species (Black et al. 2005). The procedure is based on the tendency of environmental variability to synchronize growth such that the calendar years of relatively narrow or wide growth increments correspond among individuals from a given site. By crossmatching these synchronous growth "bar codes", all growth increments can be correctly identified and thus assigned the correct calendar year. For ageing purposes, crossdating is an entirely visual process and requires no measurements or statistical analysis. The technique is similar to annual growth increment counting with the additional step of checking that growth patterns align with those in other individuals. If an increment has been missed or falsely added, the growth pattern will be offset by a year relative to that in other samples, and the point where the offset begins indicates the location of the error. In this manner, an accurate age may be obtained by working from the known year of capture at the margin through the innermost year of recruitment.

To date, crossdating has been demonstrated in a variety of aquatic species, including Pacific rockfish, freshwater mussels, and marine bivalves, including Pacific geoduck, and has been primarily used to build annually resolved chronologies for reconstructing climate or assessing the effects of climate on growth (Strom et al. 2004; Black et al. 2005; Helama et al. 2006). Yet its potential as an age validation tool has been much less thoroughly explored, even though crossdating could provide highly accurate age estimates for a large number of individuals and with no additional equipment requirements beyond a microscope. This is in contrast with most other age validation techniques, which can only accommodate small samples sizes. Under such circumstances, growth increment interpretation is validated only for a specific group of age readers in a specific group of specimens. Error could occur as the ageing criteria are employed by new age readers or applied to new samples (Beamish and McFarlane 1983; Campana 2001). Crossdating, however, validates the age estimate for each individual to which the procedure has been applied, potentially yielding large volumes of validated age data to develop high-resolution estimates of population age structure and recruitment patterns.

In this analysis, we demonstrate how crossdating can be implemented on a production-ageing level using Pacific geoduck (*Panopea abrupta*), a marine bivalve mollusk that can live in excess of 150 years and supports a multimillion dollar fishery along the Washington, British Columbia, and southeast Alaska coasts (Goodwin and Pease 1991; Orensanz et al. 2004; Strom et al. 2004). Our objective is to demonstrate the accuracy and utility of crossdating by (*i*) ageing 432 geoduck individuals via crossdating, (*ii*) statistically verifying crossdating accuracy and quantifying the strength of the synchronous growth pattern, (*iii*) evaluating the accuracy of growth increment counts versus crossdated ages, (*iv*) estimating crossdating precision by comparing geoduck ages independently crossdated by two readers, and finally (*v*) developing a highly accurate recruitment history using the crossdated data set. This study represents the first application of crossdating to generate age data, the first comparison of crossdating with growth increment count data and, to our knowledge, the first highly accurate and fully validated ageing data set for any fish or shellfish species.

Materials and methods

Pacific geoduck (*P. abrupta*) range from Kodiak, Alaska, to southern California and can be found buried in ocean floor sediment from the lower intertidal to depths up to 100 m (Bernard 1983; Coan et al. 2000). For this study, we use geoduck harvested from three sites within the Tree Nob Islands, located west of Prince Rupert, British Columbia (site 1: 54°12.587′N, 130°49.521′W; site 2: 54°13.042′N, 130°47.36′W; and site 3: 54°15.676′N, 130°48.609′W). These samples were all live-collected during density surveys conducted by commercial geoduck harvesters in the summer of 2004. Sampling depths ranged from 6 to 20 m, and divers attempted to sample nonselectively from the entire range of geoduck sizes. For crossdating and chronology development, we analyzed the growth increments found in the hinge plate. The hinge plate (inner shell layer) grows at a rate proportional to that of the outer shell layer, but is protected from erosion and thus provides the most complete record. To prepare growth increments for analysis, a single shell from each geoduck specimen was cut along the height axis of the valve through the center of the hinge plate region using a diamond-blade lapidary saw. The cut surface was polished with 600-grit sandpaper and then etched with 2% hydrochloric acid. An impression (acetate peel) was made by pressing the etched surface of the hinge plate against a piece of acetate film softened with a drop of acetone. The peel was sandwiched between two glass slides and viewed either by a microprojector or a dissecting microscope, both using transmitted light.

All 432 acetate peels were first aged by annual growth increment counts as part of routine production ageing at the Fisheries and Oceans Canada Fish Ageing Laboratory, Nanaimo, British Columbia. Subsequently, visual crossdating was conducted for 167 of the peels at the Hatfield Marine Science Center in Newport, Oregon, and for 325 peels at Nanaimo. Sixty of the same peels were crossdated at both locations to evaluate crossdating precision. The process of visual crossdating involved matching the high-frequency, year-to-year variability that occurred among specimens (Stokes and Smiley 1996). Examining high-frequency variability allowed for much more exact placement of a sample in time than would be possible using low frequency, long-term trends and also removed age-related growth declines from consideration.

In this study, we applied the list year technique in which calendar years of conspicuously narrow and wide increments

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were recorded from the marginal growth increment, formed at the known year of death (2004), to the origin (Yamaguchi 1991). The width of each growth increment was assessed relative to the widths of the two or three growth increments on either side of it. Thus, working from the margin toward the center, we noted the calendar year of each growth increment that was prominently narrow or prominently wide relative to its immediate neighbors. Applying such a narrow 5–7 year window ensured that we would record only high-frequency patterns of variability, filtering out age-related growth declines or any other low-frequency patterns.

The crossdating process was best accomplished by starting with the clearest individuals. As each new sample was processed, we checked that lists of calendar years corresponded among the other samples. If a growth increment had been missed or falsely added in the sample, recorded calendar years would be offset relative to those recorded for the other samples, and the point at which the shifting began indicated the location of the error. At no time, however, was a growth pattern forced on an individual. Dating was adjusted only when further visual inspection revealed that a growth increment had indeed been missed or falsely added. We found that we had sufficiently memorized the synchronous growth pattern after listing signature years for approximately 20 individuals and could visually crossdate the remaining samples from memory.

Of all the peels examined, 33 were particularly old and consistently clear, which we used for crossdating verification. Under the dissecting microscope and at 60× magnification, we took four to eight overlapping digital photographs with a Leica DC300 7.2 megapixel camera and used Image Pro Plus 6.0 to tile the photographs into a single panoramic image (Media Cybernetics Inc., Silver Spring, Maryland). Using Image Pro Plus 6.0, we measured growth increment widths along continuous transects that followed the axis of growth. Each growth increment was delineated at the end of the winter line and start of the new growing season. Two transects were measured on each hinge plate. To statistically validate crossdating, all 66 measurement time series were then imported into the International Tree-Ring Data Bank Dendrochronology Program Library program COFECHA written in 1982 by Richard Holmes of the Laboratory of Tree-Ring research at the University of Arizona (available through the University of Arizona Laboratory of Tree-Ring Research Web site www.ltrr.arizona.edu/software.html). In the first step of statistical validation, we used COFECHA to remove low-frequency variability in each geoduck measurement time series via the process of detrending. For this procedure, each geoduck measurement time series ($n = 66$) was fit with a cubic spline set at a 50% frequency response of 22 years (Cook and Peters 1981; Grissino-Mayer 2001). The frequency response is analogous to the length of a window used in calculating a moving average whereby the flexibility of the function increases with decreasing window length (Grissino-Mayer 2001). Cubic splines with a frequency response of 22 years provided optimal crossdating verification results in long-lived Pacific rockfish and were therefore applied in this study to Pacific geoduck (Black et al. 2005). Once fitted, each geoduck measurement time series was then divided by the values predicted by the cubic spline, thereby removing low-frequency variability, homogenizing variance, and equally weighting each set of measurements to a mean of one (Holmes 1983; Grissino-Mayer 2001). Detrended geoduck measurement time series were tested for any remaining autocorrelation, which if present was removed to ensure serial independence. Finally, each detrended measurement time series was correlated with the average of all other ($n = 65$) detrended measurement time series in the sample set. In so doing, the high-frequency growth pattern of each individual was compared with the high-frequency growth pattern of all other individuals. Isolating only the high-frequency, serially independent growth pattern prevented spuriously high correlations among individuals and also mathematically mimicked the process of visual crossdating by eliminating low-frequency growth patterns.

Results and discussion

Crossdating in geoduck

Geoduck samples exhibited a strong synchronous climate signal with several signature growth patterns that facilitated crossdating. For example, a conspicuously wide 1998 and narrow 1994 were distinctive as well as an extremely narrow 1965, a narrow 1956 (Fig. 1), a wide 1941 and narrow 1940, a narrow 1926 and 1933, a wide 1915, and a narrow 1904 and 1891. Thus at least one prominent signature year or pattern occurred approximately every decade, which allowed for frequent verification as we moved through the growth pattern. Somewhat less pronounced, yet consistently synchronous growth patterns guided crossdating between these highly visible marker years. At no time did we find a truly missing annual growth increment, though very small "micro" increments occurred in some samples, particularly in 1965. Checks, or nonannual growth zones that could be mistaken as annuli (winter zones), were much more common and quickly identified through visual crossdating. In addition to width, we used other morphological characteristics of the growth increments to aid in crossdating (Stokes and Smiley 1996; Hendy et al. 2003). For example, checks consistently occurred in 1940, and the combination of a wide 1941 and narrow 1940 with checks was highly distinctive. Once the synchronous growth pattern for the site was committed to memory, crossdating proceeded rapidly and we found we could crossdate approximately 15–20 individuals per hour.

Although crossdating procedures closely follow those developed for trees, a major difference for geoduck was that conspicuously wide signature years were just as reliable as conspicuously narrow signature years. Dendrochronologists typically rely most heavily on narrow signature years because tree growth tends to be most synchronized when climatic conditions are limiting. Almost all trees will be forced to produce a narrow ring during poor years, but growth during favorable years is often much more variable (Stokes and Smiley 1996). During a favorable year, the most competitive individuals grow vigorously while suppressed trees or those on poor microsites may fail to produce such a relatively robust increase in ring width. Such a range of growth rates often decreases synchrony during favorable years compared with that during poor climatic years. However, an analogous phenomenon is not apparent for geoduck at Tree Nob. Years with wide growth incre-

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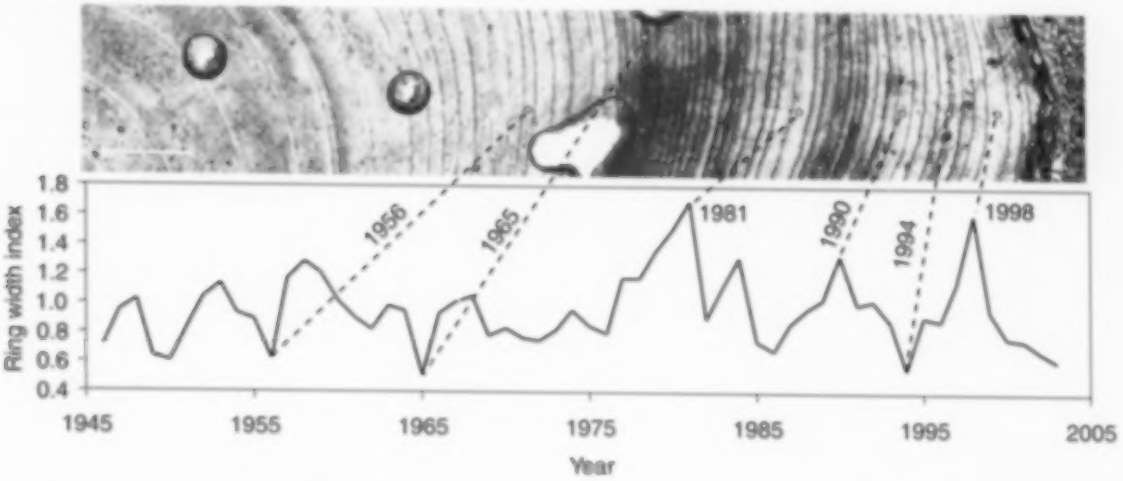
Fig. 1. Growth patterns in a geoduck (*Panopea abrupta*) hinge plate acetate peel compared with the average growth pattern for all geoducks from the Tree Nob sites, as calculated using the crossdating program COFECHA. The most common signature years (conspicuously narrow or wide growth increments relative to the two or three adjacent rings on either side) are highlighted. Note the close correspondence in growth patterns, including more subtle patterns of variability between major signature years. This geoduck specimen was harvested in the spring of 2004. Scale bar = 0.5 mm.



ments, such as 1998, 1941, and 1915, were often the most conspicuous and readily identifiable and were therefore treated the same importance as narrow years in the crossdating process. This also appears to be true in splitnose rockfish (*Sebastes diplopora*), in which wide years were also highly useful for crossdating (Black et al. 2005).

Statistical validation of crossdating verified that each growth increment had been assigned the correct calendar year and also quantified the strength of the synchronous growth pattern. Mean correlation between each set of detrended geoduck measurements and the average of all other detrended measurements (interseries correlation) was quite high at 0.735. All correlations were statistically significant ($p < 0.01$) with no segments flagged by COFECHA to check for potential errors. If, however, we simulate an error by intentionally skipping the narrow 1965 in sample No. 3a, the correlation between that sample and the average of all others drops from 0.81 (when correctly crossdated) to 0.22 (when omitting 1965), illustrating the power of this analysis. Overall, the strength of the synchronous growth pattern, as indicated by the interseries correlation, was higher than that found in mountain hemlock (*Tsuga mertensiana*) and Sitka spruce (*Picea sitchensis*) tree-ring data sets developed in the Gulf of Alaska region (Wiles et al. 1998; Gedalof and Smith 2001) and thus was at a level considered fully acceptable by dendrochronologists. The interseries correlation for geoduck was also higher than that found in Pacific rockfish (Black et al. 2005) and other marine and freshwater bivalves (Helama et al. 2006). As an additional check of accuracy, we correlated the geoduck master chronology generated by COFECHA with annual average sea surface temperature records (1941–1990) at the nearby Langara Lighthouse (54 9.0′N, 133 1.8′W). A correlation ($r = 0.72$; $p < 0.001$) was consistent with strong relationships found between geoduck growth increment width and sea surface temperatures in Puget Sound (Strom et al. 2004) and corroborated the annual periodicity of growth increment formation and annual accuracy of the chronology. Indeed, the synchronous growth pattern in geoduck appeared to be remarkably strong and consistent, which was reflected by the ease with which crossdating could be performed in this sample set.

Growth increment count ages vs. crossdated ages

When ages derived from growth increment counts were compared with crossdated ages, 89 of the 432 agreed. Among the remaining 343 individuals, 32 were over-aged by growth increment counting, while the other 311 were under-aged, in one case by 34 years (Fig. 2). The degree of underaging bias increased with geoduck age, such that growth increment count ages of the oldest individuals were the least reliable (Fig. 3*a*). This effect was particularly pronounced in geoducks that were more than 100 years in age (Fig. 3*a*). When expressed as number of underageing errors per decade, the error rate was not constant, but increased with geoduck age such that more growth increments were missed per decade in the oldest individuals than in their younger counterparts (Fig. 3*b*). This compounding error rate resulted in particularly low accuracy for older geoduck individuals. Such error could be partially explained by the fact that growth-increment width decreased with increasing age, particularly near the margin. These narrow increments were occasionally distorted, unclear, and therefore relatively difficult to interpret, leading to rapidly accumulating error. Furthermore, some hinge plates chipped during the cutting process, resulting in the loss of growth increments along the margin. The number of increments thus affected would have been greatest in older individuals, given the very slow growth rates they typically experienced late in life. During crossdating, however, missed annual growth increments or checks had the effect of offsetting the expected growth pattern and were therefore quickly identified. Even for those individuals with particularly troublesome margins, validation

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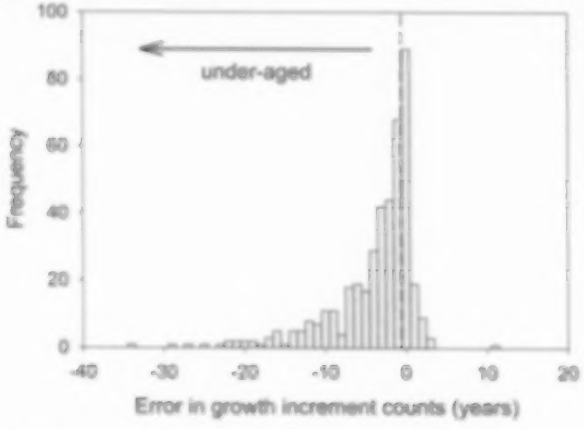
Fig. 2. Frequency distribution of the difference between growth increment count ages and crossdated ages for 432 geoduck (*Panopea abrupta*) individuals. Negative values represent underageing in growth increment count data.



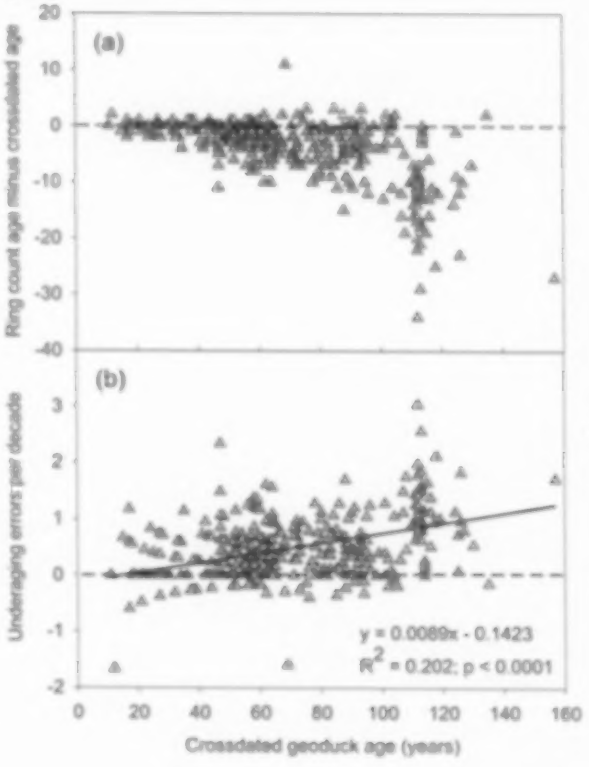
through crossdating was possible once the clear innermost growth increments were reached. In almost every sample, the inner increments formed during young, rapid growth (the first 30–50 years) were quite clear, allowing for crossdating to the origin.

Once compiled, the crossdated geoduck ages revealed a highly episodic recruitment pattern with particularly strong events in 1892–1893 and 1957–1958 (Fig. 4*a*). Apart from these two major pulses, elevated levels of recruitment occurred during the 20-year period from 1940 through 1960, but remained low throughout the remainder of the chronology (Fig. 4*a*). Although the annual growth increment count data captured recruitment from 1940–1960 and 1957–1958, the entire 1892–1893 episode was lost. Instead, underageing of the oldest samples indicated a false pulse of recruitment from 1900 to 1910 (Fig. 4*b*). In general, annual growth increment count data underestimated the highly irregular nature of geoduck recruitment at the Tree Nob sites. Recruitment pulses were much stronger and more infrequent than the growth increment count data suggested with major recruitment events separated by more than 60 years. Others have noted the episodic nature of geoduck recruitment (Orensanz et al. 2004), yet these estimates have been based on annual growth increment count data. If the results from Tree Nob are an indicator, the rarity and intensity of recruitment pulses for this species is more pronounced than previously recognized. This is particularly relevant to the management of the British Columbia geoduck fishery, which is based on an age-structured yield model where the recruitment time series reconstructed from age data has a large effect on modeled harvest rates (Zhang and Hand 2006).

Remaining error

Although crossdating can substantially increase ageing accuracy beyond that possible with growth increment counts, some error may still occur, especially when interpreting the growth increments formed during the first few years of life. In the first possible source of error, age may be underestimated by a year or two if the hinge plate center, and

Fig. 3. (*a*) Difference between the growth increment (ring) count age and the crossdated age for each of the 432 geoduck (*Panopea abrupta*) individuals, plotted against crossdated geoduck age. Negative values represent underaging in growth increment count data; a value of zero (broken line) indicates no difference between growth increment count and crossdated ages. (*b*) Underageing errors per decade, calculated as crossdated age minus growth increment count age divided by crossdated age in decades (i.e., crossdated age divided by 10). A value of zero (broken line) indicates no difference between growth increment count and crossdated ages.



thus the innermost growth increment(s), was missed during the sectioning process. Similarly, underageing may also occur if the inner shell layer (hinge plate) underwent dissolution. We cannot quantify the degree of these error sources in our data set, but believe it to be relatively low.

In another source of error, accuracy may be reduced if the innermost growth increments are unclear or ambiguous. As the origin is approached, fewer and fewer increments are available to verify the synchronous growth pattern, and consequently a mistake in these "early" growth increments cannot be readily identified through crossdating. Because of this second source of error, 23% (14 of 60) geoduck specimens independently crossdated by two readers disagreed. However, these differences were relatively minor, with 11 ages disagreeing by 1 year, two ages disagreeing by 2 years, and one age disagreeing by 3 years. The majority of this error was probably due to one reader's inexperience in interpreting the innermost growth increments. Finally, when the number of samples becomes very small (<5), as is the case

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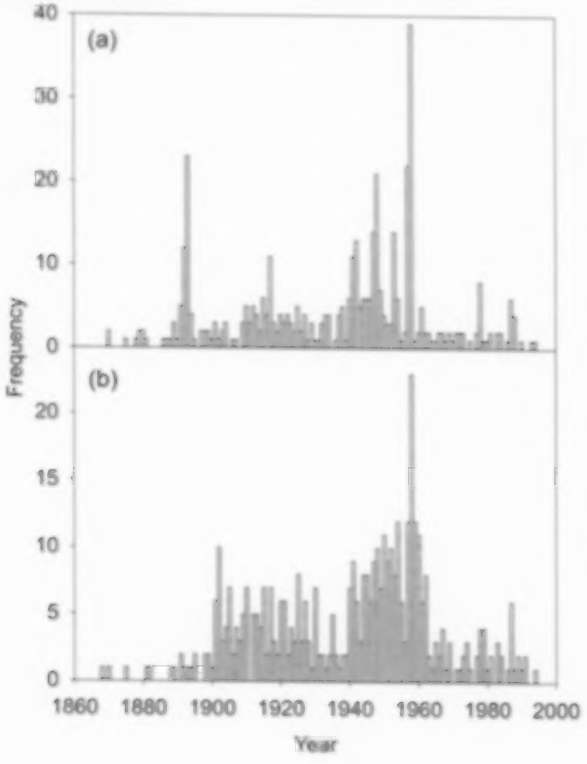
Fig. 4. Comparison between the (*a*) recruitment pattern based on crossdated ages of 432 geoduck (*Panopea abrupta*) individuals and (*b*) recruitment pattern based on growth increment count ages for the same individuals.



for the earliest growth increments in the oldest specimens, there are fewer individuals with which to correctly distinguish a true increment from a particularly convincing check or microgrowth increment. In such a circumstance, the chances of missing or falsely adding a growth increment to all samples are highest. Yet even with these sources of error, all of which would also occur in annual growth increment count data, crossdating provides a substantial improvement in accuracy as well as precision. For growth increment count data, agreement between readers in the Tree Nob data set was 19% to the same age and 48% plus or minus 1 year. By implementing crossdating, precision increased to 77% agreement to the same age and 95% plus or minus 1 year, underscoring the method's repeatability.

Applications and future directions

In conclusion, crossdating can be applied to develop highly accurate and replicable age data. The procedure has fewer requirements for specimen quality than annual growth increment count assessments and provides an objective means by which to account for all growth increments. Accurate ages can be derived even if the annual increments at the margin are missing or if there are periods of suppressed or distorted growth elsewhere in the specimen. Additional time must be invested in identifying and learning the synchronous growth pattern for the site, yet once this growth pattern is committed to memory, crossdating proceeds quickly. It can be completed more rapidly than annual growth increment counts and with greater accuracy, especially in older specimens. Crossdating also provides objective feedback for learning to correctly distinguish annual growth increments, thereby increasing the speed and ease of crossdating over time. Finally, no additional equipment beyond a microscope or microprojector is required for crossdating. The synchronous growth pattern can be described entirely through the list year technique or a related method known as skeleton plotting, both of which require no measurements (Stokes and Smiley 1996). Measurements of geoduck growth increments were completed in this study for additional verification of crossdating and as a way to quantify the strength of the synchronous growth pattern. Yet even when these statistical analyses are conducted, correlations are only a guide, and the ultimate decision as to whether a sample is correctly crossdated should be based on a visual assessment (Grissino-Mayer 2001). Computer software may help to better automate the process in the future, though technologies for pattern recognition or accurately delineating growth increments are not yet that advanced.

In future studies, a given geoduck chronology could be used to crossdate samples collected from the site at a later date or to re-evaluate historical samples. It may also be relevant for crossdating samples collected from nearby sites, particularly if the sites share a similar climate regime. Although not important at Tree Nob, visual cues other than growth increment width may also be useful to consider in future crossdating studies. For example, in X-ray exposures of coral cross sections, luminance profiles proved extremely useful in crossmatching growth patterns (Hendy et al. 2003). Analogous morphological attributes in geoduck might include prominent winter zones (annuli), uniquely transparent or opaque zones, or the presence of distinctive checks, all of which could facilitate crossdating if they occur synchronously in a given sample set. As crossdating is applied over a wider geographic range, spatial patterns in recruitment will be better resolved, as will the environmental drivers of recruitment. Higher ageing accuracy will also allow for better estimations of the coherence of geoduck recruitment and better comparisons with annually resolved environmental indices, such as sea surface temperatures, the El Niño Southern Oscillation, or the Pacific Decadal Oscillation. Given the success of crossdating in other marine and freshwater fish and shellfish species (Black et al. 2005; Helama et al. 2006), the approach will almost certainly have relevance for ageing beyond Pacific geoduck.

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Atlantic cod (*Gadus morhua*) from cold and warm environments differ in their maximum growth capacity at low temperatures

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Abstract: Maximum growth rate of Atlantic cod (*Gadus morhua*) was determined at a range of temperatures representative of cod benthic habitats (1–13 °C) using wild animals obtained from a cold environment (southern Gulf of St. Lawrence stock, SG) and a mild environment (Bay of Fundy stock, BF). SG cod performed well over a broader range of temperatures than BF cod. Growth in mass was greater for SG than BF cod at any temperature below 7 °C. SG cod consumed twice as much food as BF cod at 1 and 3 °C. At 1 °C, growth in mass was positive for SG cod but negative for BF cod, whereas growth in length did not differ from 0 for both SG and BF cod. Liver size represented a larger proportion of body mass in SG cod at both low and high temperatures, and citrate synthase activity in the intestine exhibited a strong inverse relationship to temperature. The maximum growth capacity of SG cod was comparable with that of cod of similar size in various other stocks in the Atlantic. These observations are not consistent with the view that intensive size-selective fishing practices have resulted in a significant loss of innate growth capacity in SG cod.

Résumé : La taux de croissance maximum de morues franches sauvages provenant d'un milieu froid (stock du sud du Golfe Saint-Laurent, SG) et d'un milieu tempéré (stock de la Baie de Fundy, BF) a été déterminé à des températures représentatives des habitats benthiques de la morue (1–13 °C). SG a connu une bonne croissance sur une plus large gamme de températures. Sous les 7 °C, la croissance en masse de SG a été supérieure à celle de BF. SG a consommé deux fois plus de nourriture que BF à 1 et 3 °C. À 1 °C, la croissance en masse a été positive pour SG et négative pour BF alors que la croissance en longueur a été nulle pour les poissons des deux stocks. Chez SG, la masse du foie représentait une plus forte proportion de la masse du poisson, tant aux basses qu'aux hautes températures, et l'activité de la citrate synthase dans l'intestin a nettement augmenté à basse température. La capacité de croissance maximale des morues de SG est comparable à celle des autres stocks de l'Atlantique. Ces résultats vont à l'encontre de la thèse voulant qu'une exploitation intensive et sélective des individus à forte croissance ait résulté en une réduction de la capacité innée de croissance des morues du sud du Golfe Saint-Laurent.

Introduction

The growth rate of fish varies among species and within species among individuals as a result of differences in their genetic makeup and innate capacity for growth under different sets of ecological and environmental conditions. Growth conditions vary seasonally, annually, and spatially across a species range resulting in very different growth trajectories over the life span of individual fish. This variability is well illustrated in Atlantic cod (*Gadus morhua*), a commercially exploited groundfish species inhabiting diverse environments and widely distributed in the subarctic and temperate waters of the North Atlantic (International Council for the Exploration of the Sea (ICES) 2005). Growth rate in cod varies among individuals at all stages of their life cycle (Hawkins et al. 1985; Peck et al. 2004), with growth during the early months or years having a lasting influence on sizes-at-age over life (Krohn and Kerr 1997; Armstrong et al. 2004). Growth in cod also varies seasonally (Mello and Rose 2005) and from year to year with or without a trend in the time series (Jorgensen 1992; Dutil et al. 1999).

Atlantic cod form discrete populations with a large number of stock units managed separately. These stocks differ in many respects, including mean size-at-age: for instance, by age 4, cod have barely grown to 40 cm in the Gulf of St. Lawrence, whereas they reach, on average, well over

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60 cm on Georges Bank and in the Irish Sea (Dutil et al. 1999; Armstrong et al. 2004). Growth production on a per capita basis varies sevenfold between poorly and highly productive stocks in the North Atlantic (Dutil and Brander 2003). Given the wide distribution of the species and diversity of environmental conditions across its range, marked differences in growth rates are often ascribed to divergences in environmental conditions, particularly habitat temperature. Temperature may limit growth through its effects on metabolism, and hence cod living in cold environments, such as in the Gulf of St. Lawrence, are expected to achieve in their natural environment slower growth rates than cod living in milder environments, such as in the Celtic and Irish seas (Brander 1995; Dutil and Brander 2003). Different stocks, however, may have become adapted to life in their respective environments. For instance, cod living in the southern Gulf of St. Lawrence have a higher standard metabolic rate and are not as good sprinters as cod living south in the Bay of Fundy (Sylvestre et al. 2007). Aerobic capacities are generally enhanced in cold environments, possibly explaining the elevated maintenance costs in cold environments (Lannig et al. 2003).

How much of the observed variability in growth among stocks may be due to variations in fitness and interactions between genotype and environmental conditions is largely unknown (Jónsdóttir et al. 2002; Imsland and Jónsdóttir 2003). Laboratory experiments in which larvae from different stocks are reared under rigorously similar conditions (common garden experiments) have confirmed that Atlantic cod from different populations may differ in growth capacities under strictly identical rearing conditions (Svåsand et al. 1996; Purchase and Brown 2000, 2001). The present study followed a different approach and compared the growth capacity of subadult and adult cod sampled from two stocks after having grown under natural conditions and having survived natural selective forces in their respective environments. Bay of Fundy – Western Scotian Shelf and southern Gulf of St. Lawrence cod were compared in a common garden experiment and under a range of temperatures representative of the large spectrum of bottom temperatures to which cod are exposed over the species range. The southern Gulf of St. Lawrence stock is poorly productive (Dutil and Brander 2003), has been severely depleted, and continues to be at risk in spite of reduced fishing mortality (DFO 2008). Sizes-at-age for that stock are smaller than for any other stock and, in part, may reflect a decline in the innate capacity for growth as a result of intensive fishing and selective removal of faster-growing individuals (Hanson and Chouinard 1992; Kristiansen and Svåsand 1998; Sinclair et al. 2002). The growth capacity of cod from these stocks was compared in feeding trials in which fish were fed to satiation. The growth rate of cod in the southern Gulf was hypothesized to be less than that of the more productive Bay of Fundy – Western Scotian Shelf at similar temperatures. The growth capacity was also compared with growth rates reported in earlier studies for the same stocks and in recent studies for other stocks.

Materials and methods

Atlantic cod were obtained from two stocks, the southern Gulf of St. Lawrence (SG) and the Bay of Fundy – Western Scotian Shelf (BF). The fish were caught in the Miscou Bank and Shediac Valley areas (2001 and 2002) in the southern Gulf and in the Grand Manan (2001) and Cape Sable (2002) areas on the Scotian Shelf. Fish were trawled (SG and BF) in September 2001 and were either trawled (SG) or caught by handline (BF) in June 2002. SG fish were kept overnight in land-based facilities at the Centre aquicole marin de Grande-Rivière (QC). In Grand Manan, fish were kept in 1 m^3 insulated containers on the carrier for up to 2 days, whereas in Cape Sable, land-based facilities were used before transportation. Travel by truck from the Centre aquicole in the Baie des Chaleurs area took 3–4 h, whereas from Grand Manan and Cape Sable, it took as much as 18 h. During transportation, seawater was aerated and cooled by adding ice periodically. Temperature was always maintained below 10 °C. Only one or two fish died during any transportation.

From their arrival at our facility until the experiments started, cod from the two stocks were kept in separate 7 m^3 tanks, one for each stock, and were kept under a natural photoperiod for our latitude (48°N). They were fed frozen capelin (*Mallotus villosus*) twice per week and were treated twice with a methylene blue bath (2 mg·L^{-1}, 90 min) and a formaldehyde bath (0.2 mg·L^{-1}, 60 min). During that period, salinity and temperature averaged 27.6 ± 0.2 psu and 5.3 ± 0.2 °C, respectively, before the 2001 experiment and 26.9 ± 0.8 psu and 8.2 ± 0.9 °C, respectively, before the 2002 experiment. Temperature actually declined from 6.5 °C initially to 3.2 °C when the first experiment started and increased from 7.7 °C initially to 8.7 °C when the second experiment started. Captivity conditions and growth experiments were approved by our local animal care committee and were exactly the same for both stocks, as described below.

Two experiments were conducted, one started in mid-November 2001 and lasted for 57 days using the fish caught in September 2001, and the other started in mid-August 2002 and lasted for 59 days using the fish caught in June 2002. The first experiment compared growth at 1, 3, 5, and 7 °C. The second experiment compared growth at higher temperatures (7, 9, 11, and 13 °C). Prior to the experiment, fish were anaesthetized in a bath of benzocain, prepared from 30 mL of stock solution of 0.1% benzocaine in 96% ethanol in 60 L seawater. Length (±1 mm) and mass (±1 g) were measured and fish were passive integrated transponder (PIT) tagged above the lateral line near the first dorsal fin. During the experiments, fish were fed once daily, seven days per week, with frozen capelin (five days) and northern shrimp (two days). Vitamins (Seatabs® for fish; Pacific Research Laboratories Inc., Vashon, Washington) were added to the feed once or twice per week. For each meal, fish were fed ad libitum for 1 h and then excess food was removed. Mortalities were recorded. Food consumption was determined as the difference in wet mass between food distributed and excess food removed. Total food consumption during the experiment was determined as the sum of daily food intake per tank and was expressed as food efficiency (wet mass basis, %):

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$$\text{food efficiency} = \frac{\text{change in fish biomass}}{\text{food consumed}} \times 100$$

The setup consisted of 16 circular tanks (diameter 1.5 m × depth 1 m). These were arranged as four series of tanks, each series corresponding to one of the four experimental temperatures. For each temperature, two tanks were used for SG cod and two tanks for BF cod, each tank being assigned randomly to either stock. Each tank received 7 to 10 fish. Natural seawater was used, and part of the flow was recirculated through sand filters to achieve a better control on temperature in each series of tanks. Salinity averaged 28.5 ± 0.8 psu (27.0–30.3) and 27.7 ± 1.0 psu (25.6–29.8) in experiments 1 and 2, respectively. Actual temperatures were very close to target temperatures: 1.11 ± 0.28 (1 °C), 3.08 ± 0.16 (3 °C), 4.99 ± 0.17 (5 °C), and 7.15 ± 0.29 (7 °C) in the first experiment; 6.97 ± 0.22 (7 °C), 8.94 ± 0.27 (9 °C), 11.06 ± 0.45 (11 °C), and 12.98 ± 0.14 (13 °C) in the second experiment. Differences in mean temperature among tanks within each series were less than 0.05 °C. Flow was maintained at 10 L·min^{-1} to keep dissolved oxygen above 80% saturation, including in the postprandial period, to avoid oxygen-limited growth conditions (Chabot and Dutil 1999).

Length and mass were measured at the end of the experiment 48 h after the last meal was distributed. Specific growth rate (SGR; %·day^{-1}) was calculated as

$$\text{SGR} = \frac{\text{Ln (final length)} - \text{Ln (initial length)}}{\text{time (days)}} \times 100$$

The same formula was used to calculate growth in mass. The fish were dissected to determine sex and mass of liver, pyloric caeca, intestine (from below the pylorus up to the anus), gonads, and head-off gutted carcass. Samples were taken, frozen in liquid nitrogen, and stored in a freezer at –80 °C for later analysis of protein (muscle) and lipid (liver) contents and for citrate synthase (CS) and cytochrome *c* oxidase (CCO) assays in the pyloric caeca and intestine.

Total protein content was determined using the bicinchoninic acid method (Smith et al. 1985) following tissue homogenization in KCl (150 mmol·L^{-1}) (BCA-1 kit; Sigma-Aldrich Co., St. Louis, Missouri). Total lipid content was determined using Reisenbichler and Bailey's (1991) microextraction method followed by a microgravimetric determination. Moisture content in the liver was determined from mass loss of a 10 g sample over 48 h in an oven set at 65 °C and was used to report liver lipid (determined on dry samples) per gram of wet mass. Twenty fish from each stock were sacrificed at the start of each experiment to obtain base values for the same parameters. Protein and lipid contents of the food (capelin and shrimp) were determined periodically, as described above, and capelin and shrimp consumption were expressed as protein and lipid ingestion per fish per tank. Carcass protein was determined as the product of carcass mass and muscle protein concentration on a wet mass basis, and liver lipid was determined as the product of liver mass, liver moisture content, and liver lipid concentration on a wet mass basis (averaged over all fish in a tank). Cytochrome *c* oxidase and citrate synthase activity of the pyloric caeca and intestine were determined following Thibault et al. (1997). Samples of intestine and pyloric caeca (~0.5 g) were homogenized in imidazole buffer (50 mmol·L^{-1} imidazole, 2 mmol·L^{-1} $MgCl_2{\cdot}6H_2O$, 5 mmol·L^{-1} EDTA, 0.1% Triton X-100, and 1 mmol·L^{-1} reduced glutathione at pH 7.5). Phenylmethylsulfonyl fluoride (PMSF, 0.1 mmol·L^{-1} for intestine and 1 mmol·L^{-1} for pyloric caeca) was added to the imidazole buffer. Tissues were disrupted in three 20 s bursts of homogenization (Tekmar tissue grinder; Tekmar, Cincinnati, Ohio). Homogenates were centrifuged for 3 min at 1000*g* (4 °C) (Centrifuge 22; Heraeus Sepatech, Osterode/Harz, Germany), and the supernatant was taken for the analyses. Enzymatic assays were carried out at 20 °C. Readings were made using an ultraviolet–visible (UV–VIS) spectrophotometer (Lambda 11; PerkinElmer Life and Analytical Sciences, Shelton, Connecticut) equipped with a thermostated cell holder and a circulating refrigerated water bath. All assays were run in duplicate, and enzymatic activity was expressed in U·(g tissue)$^{-1}$ (U is 1 µmole of substrate transformed per minute). Citrate synthase was assayed in a 100 mmol·L^{-1} imidazole–HCl buffer with 0.1 mmol·L^{-1} DTNB, 0.1 mmol·L^{-1} acetyl-CoA, and 0.15 mmol·L^{-1} oxaloacetate (omitted for the control) at pH 8.0. Optical density was measured for a minimum of 3 min at 412 nm to track the transfer of sulphydril group from CoA-SH to DTNB. The extinction coefficient used was 13.6 L·mmol^{-1}·cm^{-1}. Cytochrome oxidase was assayed in a phosphate buffer (50 mmol·L^{-1}, pH 8.0) with 70 µmol·L^{-1} of reduced cytochrome *c* (from horse heart). Cytochrome *c* was reduced with sodium hydrosulfite, and the excess hydrosulfite was removed by bubbling the solution for 3 min. Optical density was followed for 3 min at 550 nm, and the extinction coefficient used was 19.1 L·mmol^{-1}·cm^{-1}. All the chemicals used for proteins and enzymatic assays were purchased from Sigma-Aldrich Co.

Statistical analyses were conducted with SAS (version 8; SAS Institute Inc., Cary, North Carolina) and SYSTAT (release 11.00.01; SYSTAT Software Inc., San Jose, California). Differences were considered significant when P was less than 5%. Values are reported as mean ± standard deviation (MSD), least-square means ± standard error (MSE), or with 95% confidence intervals (MCI). Fish length and fish weight data were log-transformed. The analyses were restricted to fish with initial lengths within the range of 38 to 56 cm. Individual fish were considered as replicates when groups at the start of the experiment were compared. Tanks were considered as the experimental units at the end of the experiment, with two random observations for each combination of stock, experiment, and temperature based on the mean value for pseudoreplicates (mean value for all fish in each tank).

Differences in length and mass at the start of the experiment between experiments and between stocks for each experiment were tested with two-sample t tests, whereas differences among treatments were tested with one-factor analyses of variance (ANOVAs) for each stock and experiment combination separately. The mass–length relationship at the start of the experiment for each stock and experiment was described with least-square linear regression on log-transformed data. Total mass for fish of a standard length (465 mm) was derived from those relationships.

Differences in SGR in length and SGR in mass between

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Table 1. Fork length (mean ± standard deviation, SD), total fish mass (mean ± SD), and number of Atlantic cod (*Gadus morhua*) at the start of the experiments (number in parentheses).

	Experiment 1		Experiment 2	
Stock	Fork length (mm)	Total mass (g)	Fork length (mm)	Total mass (g)
SG	451±33 (93)	732±188 (93)	461±32 (105)	745±154 (105)
BF	485±33 (84)	1077±255 (84)	469±39 (94)	867±248 (94)

Note: Experiment 1 compared growth at lower temperatures (1, 3, 5, 7 °C), and experiment 2 compared growth at higher temperatures (7, 9, 11, 13 °C). Atlantic cod from the southern Gulf of St. Lawrence (SG) and from the Bay of Fundy (BF) were used. These statistics include 80 cod sacrificed at the start of the experiments to obtain reference values (20 fish per stock and experiment).

experiments for the only treatment in common between experiments (7 °C) were tested with the procedure MIXED in SAS (release 8.02; SAS Institute Inc.), with stock and experiment as fixed effects and tank as a random factor nested within stock and experiment. Homogeneity of the variances was checked as follows. We calculated the difference in the log-likelihood statistic for two nested models, one based on a common variance and one based on separate variances for the two experiments. That value was compared with critical values of the chi-square distribution with the number of degrees of freedom being equal to the difference in the number of parameters in the two covariance matrix. The assumption of normality was assessed with Shapiro–Wilk's W statistic for the residuals of one of the two models, depending on whether variances were homogeneous or not homogeneous. When residuals were found not to be normally distributed, the normal density plot was examined; based on visual examination, low data skewness or kurtosis, i.e., slight deviations from normality, was ignored. Differences in SGR in length and SGR in mass between treatments were tested for each experiment separately. We followed a similar approach as described above except that fixed effects were stock and treatment and random factor tank was nested within stock and treatment. Homogeneity of the variances was checked for stock and treatment separately. Tukey's tests were used for pairwise comparisons. The same approach was used to examine food consumption and food efficiency data, as well as differences in enzyme activities and protein and lipid ingestion and contents.

The size of different tissues at the start of the experiment was determined based on the data for the fish dissected at the start of the experiments. The average tissue mass was calculated from the mean mass at standard length (465 mm) and from linear relationships between tissue mass and total mass. Condition at the end of the experiment was determined as follows. The final length of fish of standard length at the start of the experiment was calculated using the observed SGR in length for each stock, treatment, and experiment. Corresponding fish mass and tissue mass were determined from the mass–length relationship and from linear relationships between tissue mass and total mass observed at the end of the experiment for each stock, treatment, and experiment. The size of tissues relative to fish size was calculated as (mass of the tissue / total mass of the fish) × 100.

For the gonads, we used a different approach. The size of the gonads relative to fish size (RGS) was calculated as [mass of the gonads / (total mass of the fish – mass of the gonads)] × 100. Nonmaturing and maturing fish were assumed to have RGS values less than and greater than 3.0, respectively (Dutil and Lambert 2000). Variations in RGS were examined for maturing and nonmaturing fish separately, considering stock and experiment as fixed effects and fish length as a covariate in analyses of covariance (ANCOVAs). Differences in proportions of maturing and nonmaturing fish were tested with χ^2 tests.

Results

The length at the start of the experiment did not differ between experiments (46.5 ± 3.6 cm MSD; 46.7 ± 3.7 and 46.4 ± 3.6 cm in experiments 1 and 2, respectively, P = 0.52); BF cod were slightly larger than SG cod in the first experiment (by 3.4 cm, $P < 0.001$), but not in the second experiment (by <1 cm, $P = 0.10$) (Table 1). Total mass at the start of the experiment differed between experiments (by 94 g, $P < 0.001$) owing to differences in mass between experiments for BF cod. Differences between stocks within each experiment were also significant, with BF cod heavier than SG cod: 345 g and 122 g for the first and second experiments, respectively ($P < 0.001$). Length and mass did not differ among treatments for any of the stock and experiment combinations ($P > 0.05$).

Tissue mass at the start of the experiments varied with fish mass for both stocks and in both experiments ($P < 0.05$). Pyloric caeca mass represented 1% of fish mass. The mass of the intestine was smaller for SG than for BF cod, whereas the mass of the liver was larger in experiment 1 than in experiment 2. The mass of the carcass represented 67.5%–70.1% of fish mass, and this percentage increased with fish size at the start of the experiment (Table 2). Based on RGS, the majority of the fish (73 out of 80) were classified as being sexually immature at the start of the experiment. Fish length was not a significant covariate ($P > 0.05$) in any of the ANCOVAs testing for differences in RGS among stocks and experiments, both for maturing and immature fish (Table 3).

Growth rate at 7 °C was similar in both experiments. SGR in length averaged 0.1195 ± 0.0069 and 0.0901 ± 0.0070 (MSE) in experiments 1 and 2, respectively. There was a significant effect of experiment ($P = 0.04$) and no interaction between the two fixed factors (stock and experiment). SGR in mass at that temperature averaged 0.7351 ± 0.0428 and 0.6823 ± 0.0432 (MSE) in experiments 1 and 2, respectively. There was no significant effect of experiment (P = 0.43) and no interaction between the two fixed factors (stock and experiment, $P = 0.33$). Variances in SGR data were

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Table 2. Total and tissue mass (g) of Atlantic cod (*Gadus morhua*) of standard length (465 mm) at the start of the experiments.

		Stock	
	Exp.	SG	BF
Total	1	810.1	953.3
		785.9, 835.3	924.9, 982.5
	2	763.2	829.6
		748.6, 777.8	809.5, 851.1
Pyloric caeca	1	9.3 (1.1%)	10.7 (1.1%)
		8.2, 10.3	9.9, 11.5
	2	7.0 (0.9%)	8.9 (1.1%)
		6.2, 7.7	7.8, 10.0
Intestine	1	4.5 (0.6%)	7.7 (0.8%)
		4.2, 4.9	7.1, 8.3
	2	4.5 (0.6%)	6.7 (0.8%)
		4.0, 4.9	6.1, 7.3
Liver	1	24.0 (2.9%)	34.6 (3.6%)
		19.9, 28.1	28.2, 40.9
	2	14.9 (1.9%)	10.3 (1.2%)
		12.6, 17.2	8.3, 12.3
Carcass	1	556.4 (68.6%)	669.0 (70.1%)
		549.2, 560.3	653.5, 684.4
	2	515.1 (67.5%)	575.8 (69.4%)
		510.1, 520.1	568.4, 583.1

Note: Mean values are shown with 95% confidence intervals given on the line below. Tissue mass – fish mass ratios are expressed as percentages and are shown in parentheses after the means. Atlantic cod from the southern Gulf of St. Lawrence (SG) and from the Bay of Fundy (BF) were used. These statistics are based on 80 cod sacrificed at the start of the experiments (Exp.) to obtain reference values (20 fish per stock and experiment).

homogeneous both for growth in length and growth in mass data ($P > 0.05$). For growth in length, SGR data residuals followed a normal distribution ($P > 0.05$), whereas for growth in mass, SGR data residuals did not follow a normal distribution ($P < 0.05$). Because the experiments were run at a 1-year interval, the two experiments have been considered separately in further analyses of growth rate (effects of temperature).

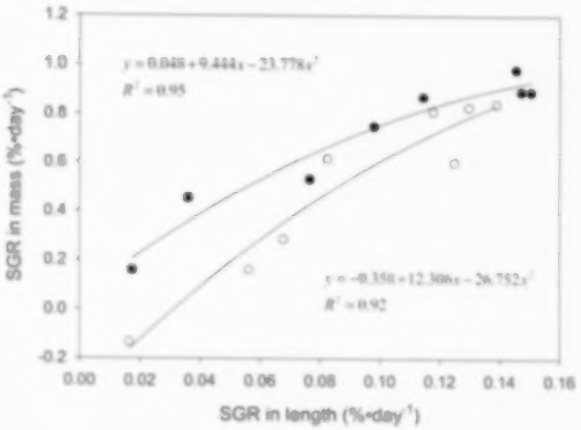
High temperatures (experiment 2) promoted faster growth rates than low temperatures (experiment 1) for both SG and BF cod, with SG cod experiencing a faster growth in mass than BF cod at comparable growth rates in length (Fig. 1). Treatment had a significant effect on SGR in length in both experiment 1 ($P < 0.001$, with all pairwise comparisons significant) and experiment 2 ($P = 0.02$, SGR in length smaller at 7 °C, other treatments did not differ significantly). Stock and the interaction term were nonsignificant in either experiment ($P > 0.05$; Table 4). Treatment also had a significant effect on SGR in mass in experiment 1 ($P < 0.001$, with all pairwise comparisons significant), but not in experiment 2 ($P = 0.16$). Stocks differed significantly in SGR in mass, with SG cod experiencing faster growth in mass than BF cod at any temperature in experiment 1 ($P < 0.05$). There was no difference between stocks in experiment 2 ($P = 0.14$), and the interaction term was nonsignificant in either experiment ($P > 0.05$; Table 4). SGR in length at 1 °C did not differ significantly from 0 for both SG ($P = 0.10$) and BF ($P = 0.16$) cod. SGR in mass was positive and greater than 0 for SG cod ($P = 0.02$), but did not differ from 0 for BF cod ($P = 0.08$).

Table 3. Mass of the gonads (g) and number of Atlantic cod (*Gadus morhua*) (in parentheses) with a gonads mass – somatic mass ratio (RGS, %) less than 3.0 (sexually immature) and greater than 3.0 (maturing fish) at the start of the experiments (Exp.).

	Stock			
	SG		BF	
Exp.	RGS < 3.0	RGS > 3.0	RGS < 3.0	RGS > 3.0
1	8.4	34.6	5.0	33.2
	5.6, 11.2	—	1.8, 8.2	27.9, 38.4
	(19)	(1)	(18)	(2)
2	8.3	47.0	6.8	
	5.7, 10.9	37.3, 56.6	3.9, 9.8	
	(16)	(4)	(20)	(0)

Note: Tissue mass was adjusted to an average length (465 mm) and corresponding fish mass (Table 2). Mean values are shown with 95% confidence intervals given on the line below. Atlantic cod from the southern Gulf of St. Lawrence (SG) and from the Bay of Fundy (BF) were used.

Fig. 1. Growth rate of Atlantic cod (*Gadus morhua*) from the southern Gulf of St. Lawrence (SG, solid symbols) and the Bay of Fundy (BF, open symbols) at different temperatures in two experiments. Experiment 1 compared growth at lower temperatures (1, 3, 5, 7 °C); experiment 2 compared growth at higher temperatures (7, 9, 11, 13 °C). Specific growth rate (SGR) in mass is plotted against SGR in length; least-square means are shown and were fit with second-order polynomial regressions.



Growth resulted from an increase in the size of all tissues examined, particularly the liver and carcass, but pyloric caeca and intestine responded differently at low temperatures (Table 5). Final liver size reflected trends in SGR in mass, with a similar relationship for southern Gulf as for Bay of Fundy cod (Fig. 2). This resulted in consistently larger livers for SG cod and increasing liver size with increasing temperature up to a plateau at high temperatures. In BF cod, liver size did not increase much at 1, 3, and 5 °C (experiment 1), whereas it increased 10-fold and represented a liver to total mass ratio of 8% at high temperatures

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Table 4. Specific growth rate (SGR; %·day^{-1}; least-square mean ± standard error) in length and in mass of Atlantic cod (*Gadus morhua*) from the southern Gulf of St. Lawrence (SG) and the Bay of Fundy (BF) at different temperatures in two experiments.

		SGR in length		SGR in mass	
Exp.	Treat.	SG	BF	SG	BF
1	1	0.0174±0.0092	0.0164±0.0105	0.1599±0.0561	-0.1330±0.0561
	3	0.0361±0.0092	0.0562±0.0090	0.4540±0.0562	0.1619±0.0548
	5	0.0765±0.0089	0.0677±0.0094	0.5319±0.0536	0.2867±0.0575
	7	0.1143±0.0087	0.1248±0.0090	0.8685±0.0525	0.6018±0.0548
2	7	0.0979±0.0128	0.0824±0.0147	0.7490±0.0876	0.6157±0.0975
	9	0.1454±0.0128	0.1176±0.0133	0.9809±0.0876	0.8102±0.0903
	11	0.1504±0.0128	0.1295±0.0136	0.8901±0.0876	0.8276±0.0918
	13	0.1471±0.0136	0.1388±0.0136	0.8916±0.0919	0.8414±0.0918

Note: Exp., experiment; Treat., treatment. Experiment 1 compared growth at lower temperatures (1, 3, 5, 7 °C); experiment 2 compared growth at higher temperatures (7, 9, 11, 13 °C).

Table 5. Total and tissue mass (g) of Atlantic cod (*Gadus morhua*) at the end of the experiments.

Stock	Exp.	Treat.	Total mass	Pyloric caeca	Intestine	Liver	Carcass
SG	1	1	896.4	16.3 (15.1, 17.5)	7.2 (6.7, 7.7)	56.4 (52.1, 60.6)	593.7 (586.1, 601.6)
		3	1028.5	17.9 (16.8, 19.0)	7.8 (7.3, 8.2)	68.5 (64.4, 72.5)	684.0 (676.8, 691.6)
		5	1137.2	19.2 (18.1, 20.4)	8.3 (7.8, 8.7)	78.4 (74.3, 82.6)	758.3 (750.9, 766.1)
		7	1328.1	21.5 (20.2, 22.9)	9.1 (8.6, 9.7)	95.9 (91.0, 100.9)	888.8 (880.1, 898.1)
	2	7	1176.6	15.6 (14.6, 16.6)	8.2 (7.7, 8.6)	103.3 (98.6, 108.1)	809.5 (815.2, 814.3)
		9	1319.4	17.7 (16.7, 18.8)	9.1 (8.7, 9.6)	123.6 (119.1, 128.2)	905.7 (911.7, 910.1)
		11	1325.2	17.8 (16.8, 18.9)	9.2 (8.7, 9.6)	124.5 (119.9, 129.0)	909.6 (915.6, 914.0)
		13	1291.2	17.3 (16.3, 18.3)	8.9 (8.5, 9.4)	119.6 (115.1, 124.2)	886.7 (892.7, 891.1)
BF	1	1	835.0	13.3 (11.6, 15.1)	8.9 (7.7, 10.1)	24.8 (17.0, 32.6)	574.6 (560.6, 588.4)
		3	1056.0	16.1 (14.9, 17.2)	9.8 (9.0, 10.7)	46.6 (41.4, 51.8)	728.9 (718.6, 738.9)
		5	1092.6	16.5 (15.4, 17.6)	10.0 (9.2, 10.8)	50.2 (45.2, 55.1)	754.4 (744.6, 764.1)
		7	1359.9	19.9 (18.9, 20.8)	11.2 (10.5, 11.8)	76.5 (72.2, 80.7)	941.1 (931.3, 950.4)
	2	7	1251.3	16.6 (15.4, 17.8)	9.2 (8.7, 9.7)	97.6 (89.3, 106.0)	850.7 (839.3, 862.6)
		9	1292.8	17.1 (16.0, 18.3)	9.5 (9.0, 10.1)	102.3 (94.1, 110.5)	878.1 (866.7, 890.0)
		11	1289.5	17.1 (15.9, 18.3)	9.5 (9.0, 10.0)	101.9 (93.7, 110.1)	875.9 (864.5, 887.8)
		13	1351.4	17.8 (16.7, 19.0)	10.0 (9.5, 10.5)	108.8 (100.8, 116.9)	916.8 (905.2, 928.8)

Note: Exp., experiment; Treat., treatment. Experiment 1 compared growth at lower temperatures (1, 3, 5, 7 °C); experiment 2 compared growth at higher temperatures (7, 9, 11, 13 °C). Mean predicted values are shown with 95% confidence intervals given in parentheses. Atlantic cod from the southern Gulf of St. Lawrence (SG) and from the Bay of Fundy (BF) were used.

(experiment 2; Tables 2 and 5). In contrast, liver size increased several-fold and represented a larger proportion of total mass at both low and high temperatures in SG cod. When liver mass (as an indicator of energy storage) is expressed as a percentage of carcass mass (as an indicator of swimming muscle), liver size is found to have increased in a greater proportion during the experiments (calculated from Tables 2 and 5): liver to carcass ratio (LCR) increased from 4.3% to 10.2% (experiment 1) and from 2.9% to 13.4% (experiment 2) in SG cod across temperatures. In BF cod, the change was dependent on temperature in experiment 1 (from 5.2% down to 4.3% at 1 °C and up to 8.1% at 7 °C), but not in experiment 2 (from 1.8% up to 11.7% across temperatures). Final pyloric caeca mass correlated significantly with SGR in mass in BF ($P < 0.05$) but not in SG ($P = 0.51$) cod and represented a larger proportion of total mass at low temperatures (1.5%–1.8%) than at high temperatures (1.3%). In contrast, the mass of the intestine correlated significantly with SGR in mass in SG ($P < 0.01$) but not in BF ($P = 0.48$) cod. The mass of the intestine also represented a larger proportion of total mass at low temperatures.

Based on RGS, a majority of the fish were sexually immature at the end of the experiment (236 out of 296; Table 6), a slightly lower proportion (0.80) compared with the situation at the start of the experiment (0.91) ($\chi^2 = 5.7$, 1 df; $P = 0.02$). A significantly higher proportion of SG cod became sexually mature in experiment 1 ($\chi^2 = 10.1$, 1 df; $P < 0.01$), whereas a significantly higher proportion of BF cod became sexually mature in experiment 2 ($\chi^2 = 29.1$, 1 df; $P < 0.001$). Treatment influenced that proportion for SG cod in experiment 1 ($\chi^2 = 8.7$, 3 df; $P = 0.03$) but not for BF cod in experiment 2 ($\chi^2 = 2.5$, 3 df; $P = 0.47$). RGS was not influenced by treatment in either case ($P = 0.44$ and $P = 0.15$ for SG cod in experiment 1 and BF cod in experiment 2, respectively). There was no significant relationship between RGS and length for any of the stock and experiment combination for either sexually mature or immature fish ($P > 0.05$).

Food consumption differed between stocks and among

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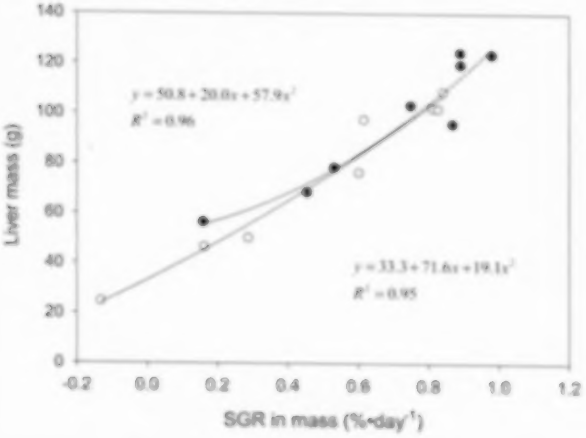
Fig. 2. Final liver size of Atlantic cod (*Gadus morhua*) from the southern Gulf of St. Lawrence (SG, solid symbols) and the Bay of Fundy (BF, open symbols) at different temperatures in two experiments. Experiment 1 compared growth at lower temperatures (1, 3, 5, 7 °C); experiment 2 compared growth at higher temperatures (7, 9, 11, 13 °C). Liver size is plotted against specific growth rate (SGR) in mass; least-square means are shown and were fit with second-order polynomial regressions.



treatments in experiment 1 ($P < 0.001$), as well as in experiment 2 ($P = 0.03$ and 0.01 for stocks and treatments, respectively). The interaction term was not significant in either case ($P > 0.05$). In experiment 1, food consumption was lower at 1 °C and greater at 7 °C than at 3 °C and 5 °C, whereas in experiment 2, food consumption was less at 7 °C than at higher temperatures ($P < 0.05$). In experiment 1, SG cod consumed twice as much food as BF cod at 1 °C and 3 °C (0.69 g·g^{-1} and 1.45 g·g^{-1} vs. 0.33 g·g^{-1} and 0.66 g·g^{-1}, respectively). In experiment 2, maximum consumption averaged 2.59 g·g^{-1} for SG cod and 2.22 g·g^{-1} for BF cod at 9, 11, and 13 °C. Food efficiency differed between stocks and among treatments in experiment 1, with a significant interaction term ($P < 0.001$). The ratio was consistently higher for SG cod, with a marked difference at 1 °C (14.4% and –23.1% for SG and BF cod, respectively) and 3 °C (21.8% and 13.7% for SG and BF cod, respectively). These results indicate that BF cod were unable to increase their mass despite an unlimited food ration at 1 °C. In contrast, no difference was observed between stocks and among treatments in experiment 2 ($P > 0.05$); food efficiency averaged 28.2% for both stocks at higher temperatures (experiment 2). The relationship between food consumption or food efficiency and growth was similar for the two stocks, with differences in growth in mass between stocks at any temperature explained by stock-specific differences in food consumption and food efficiency (Fig. 3).

Total protein in the carcass correlated with protein ingestion in the first experiment, with marked differences at low ingestion rates (coldest temperatures) becoming weak at higher ingestion rates, i.e., 7 °C in the first experiment and all temperatures in the second experiment (Fig. 4). This was consistent with observed differences in size and growth rate for SG and BF in the first experiment (Tables 2 and 4). Protein ingestion did not differ between stocks ($P > 0.05$, both experiments), but did differ among treatments ($P < 0.01$, both experiments) and increased with increasing temperature up to a plateau at 9 °C. The interaction term was not significant ($P > 0.05$, both experiments). Total protein in the carcass differed between stocks ($P < 0.001$), as well as among treatments ($P < 0.01$), and increased with increasing temperature in the first experiment. The interaction term was not significant ($P > 0.05$). In the second experiment, no significant effects of stock or treatment were observed ($P > 0.05$). There was only a slight increase in liver lipid with increasing food ingestion in the first experiment, as indicated by the low slope in the relationship between liver lipid content and food lipid ingestion (Fig. 4), indicating that food lipids were not being used exclusively for lipid storage in the liver at higher temperatures. In contrast, total liver lipid increased sharply with increased lipid ingestion in the second experiment, consistent with patterns in the initial and final liver size in both experiments (Tables 2 and 5). Lipid ingestion followed a similar pattern as protein ingestion: no differences between stocks ($P = 0.08$ and $P = 0.60$ in experiments 1 and 2, respectively) and significant differences among treatments in both experiments ($P > 0.05$). The interaction term was not significant in either experiment. In experiment 1, total lipid in the liver did not differ between stocks, but did differ among treatments: least-square mean values were 18.6^a, 25.0^b, 24.4^b, and 29.6^c g at 1, 3, 5, and 7 °C, respectively (values with same letter did not differ in Tukey's tests, $P > 0.05$). The interaction term was not significant ($P > 0.05$). In experiment 2, total lipid in the liver averaged 75.3 g, with no significant effects of stock or treatment ($P > 0.05$). When comparing the two experiments at a common temperature (7 °C), significant differences were observed in total lipid ingestion ($P < 0.001$, 162.9 and 129.9 g for experiments 1 and 2, respectively), and total liver lipid contents ($P < 0.05$, 29.5 and 68.0 g for experiments 1 and 2, respectively). There was no stock effect and the interaction term was not significant ($P > 0.05$).

The most striking result for metabolic assays was observed for citrate synthase in the intestine. Citrate synthase differed significantly between stocks in both experiments ($P < 0.01$), and enzyme activity varied with temperature in both experiments ($P < 0.01$), with a strong trend for increasing enzyme activity at lower temperatures for SG cod (Fig. 5). Citrate synthase activity was greater in cold than in warm water by 162% and 66% in southern Gulf and Bay of Fundy cod, respectively. Cytochrome *c* oxidase in the intestine was greater for SG than for BF cod in both experiments ($P < 0.04$), but temperature had no significant effect in either experiment ($P > 0.60$). Enzymatic activities responded differently in the pyloric caeaca. Citrate synthase and cytochrome *c* oxidase did not differ significantly between stocks ($P > 0.05$). Only cytochrome *c* oxidase varied across treatments in both experiments, with lower enzyme activities at the lowest temperatures within each experiment ($P < 0.05$). The interaction term was never significant ($P > 0.12$).

Discussion

Natural mortality exerts a selective pressure on fish by which smaller fish stand fewer chances of survival than larger fish of the same cohort (Henderson et al. 1988; Post

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Table 6. Mass of the gonads (g, mean values with 95% confidence intervals) and number of fish (in parentheses) with a gonads mass – somatic mass ratio (RGS, %) less than 3.0 (sexually immature) and greater than 3.0 (maturing fish) at the end of the experiments.

		Stock			
		SG		BF	
Exp.	Treat.	RGS < 3.0	RGS > 3.0	RGS < 3.0	RGS > 3.0
1	1	15.4	50.2	6.8	60.8
		10.4, 20.3	–46.5, 147.0	3.9, 9.7	—
		(14)	(3)	(11)	(1)
	3	13.4	66.1	11.7	
		10.0, 16.8	15.6, 116.6	6.5, 16.8	
		(12)	(5)	(18)	(0)
	5	19.2	40.6	13.8	60.6
		12.9, 25.6	27.0, 54.3	10.1, 17.6	—
		(14)	(5)	(15)	(1)
	7	22.3	76.8	16.5	42.9
		14.8, 29.7	60.9, 92.8	11.7, 21.3	—
		(8)	(12)	(17)	(1)
2	7	13.1	41.5	15.4	95.7
		9.6, 16.6	17.6, 65.3	7.3, 23.5	51.6, 139.9
		(20)	(2)	(8)	(8)
	9	12.2		16.3	64.1
		9.6, 14.7		7.9, 24.8	31.1, 97.2
		(22)	(0)	(14)	(6)
	11	7.9	43.9	15.1	62.4
		5.4, 10.3	—	11.9, 18.2	10.4, 114.4
		(21)	(1)	(13)	(6)
	13	8.3		18.9	79.2
		4.8, 11.8		7.7, 30.2	60.7, 97.8
		(19)	(0)	(10)	(9)

Note: Exp., experiment; Treat., treatment. Experiment 1 compared growth at lower temperatures (1, 3, 5, 7 °C); experiment 2 compared growth at higher temperatures (7, 9, 11, 13 °C). Mean predicted values are shown with 95% confidence intervals given on the line below. Atlantic cod from the southern Gulf of St. Lawrence (SG) and from the Bay of Fundy (BF) were used.

and Evans 1989). Larger fish are expected to contribute to spawning in a larger proportion and to maintain the innate capacity for fast growth in the population. Species exploited commercially may be under an opposite selective pressure: fishing may result in the selective removal of fast-growing individuals, i.e., removal of fish that recruit to the fishery at an earlier age and before becoming sexually mature, resulting in various phenotypic and genotypic responses, particularly in reproductive strategies (Fudge and Rose 2008). Studies on cod and using methods that remove mortality and growth variation effects suggest an evolutionary basis for the observed decreases in age and size at maturation in response to high fishing mortalities (Barot et al. 2004; Olsen et al. 2004). Routine surveys conducted for stock assessment purposes monitor sizes-at-age as an indicator of growth. Declining trends in sizes-at-age over time have been observed in several stocks in the past, for instance, in the northern Gulf of St. Lawrence (Dutil et al. 1999), on the Grand Banks (Krohn and Kerr 1997), off West Greenland (Hansen 1987), and in the Barents Sea (Jorgensen 1992). Whether intensive size-selective predation by harp (*Pagophilus groenlandicus*) and gray (*Halichoerus grypus*) seals (Hammill and Stenson 2000, 2002) and fishing (Hanson and Chouinard 1992; Kristiansen and Svåsand 1998; Sinclair et al. 2002) have resulted in a reduced innate capacity for growth overall and might explain smaller sizes-at-age in some stocks is still open to question (Imsland and Jónsdóttir 2002). Swain et al.'s (2007) analysis of back-calculated lengths-at-age suggested that the slower growth in southern Gulf cod had evolved in response to size-selective fishing, but their interpretation of the data has been challenged (Heino et al. 2008).

Whether maximum growth capacity of cod has changed over time in the southern Gulf of St. Lawrence cannot be determined, but our experiments have shown that southern Gulf cod in the size range used in our experiments still have an innate capacity to grow fast under nonlimiting food conditions. Their growth rate was comparable with that of cod of similar size from various other stocks, including stocks not considered to have been overexploited (Fig. 6). This observation is not consistent with a potential evolutionary basis for the observed slow growth rates for that stock during and after the decline of its abundance, suggesting that other factors may have contributed more significantly. Svåsand et al. (1996) demonstrated slight stock-specific differences in the growth performance of Arcto-Norwegian and

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Fig. 3. Food consumption (circles) and food efficiency (triangles) on a wet mass basis of Atlantic cod (*Gadus morhua*) from the southern Gulf of St. Lawrence (SG, solid symbols) and the Bay of Fundy (BF, open symbols) plotted against (*a*) temperature and (*b*) specific growth rate (SGR) in two experiments. Experiment 1 compared growth at lower temperatures (1, 3, 5, 7 °C); experiment 2 compared growth at higher temperatures (7, 9, 11, 13 °C).

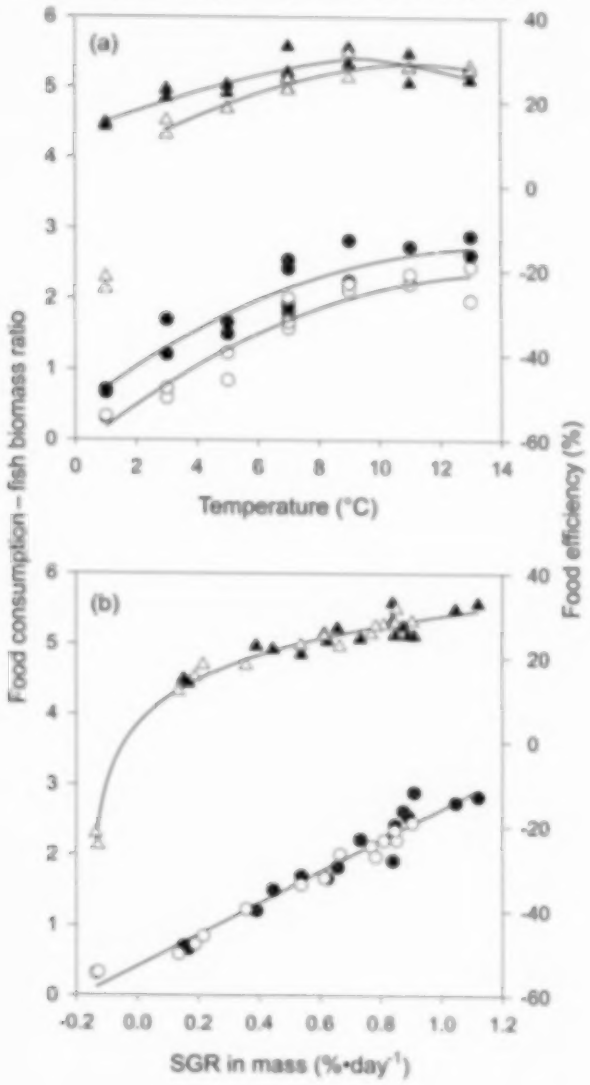
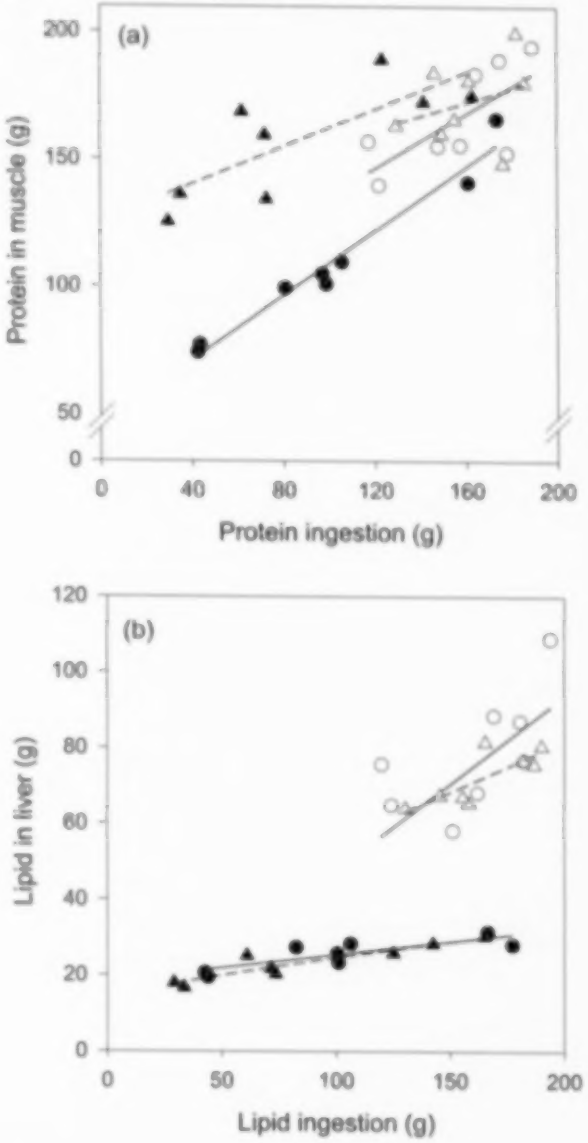


Fig. 4. (*a*) Protein contents in muscle and (*b*) lipid contents in liver in relation to protein and lipid ingestion (per fish per tank), respectively, of Atlantic cod (*Gadus morhua*) from the southern Gulf of St. Lawrence (SG, circles) and the Bay of Fundy (BF, triangles) in two experiments. Experiment 1 compared growth at lower temperatures (solid symbols; 1, 3, 5, 7 °C); experiment 2 compared growth at higher temperatures (open symbols; 7, 9, 11, 13 °C).



Norwegian coastal cod after one year under standard farming conditions. Juvenile cod from two stocks, Grand Banks and Gulf of Maine, exhibited similar growth rates in response to changing temperatures in the laboratory, suggesting that faster growth of southern cod populations in the wild was not due to a higher genetic capacity for growth (Purchase and Brown 2000, 2001). Growth production of cod averages 250 g·fish^{-1}·year^{-1} in the southern Gulf and 710 g·fish^{-1}·year^{-1} in the Bay of Fundy (Dutil and Brander 2003). One potential explanation for the slow growth production observed in the late 1980s and in the 1990s concurrently in several stocks of the Northwest Atlantic is that large-scale natural climatic events have lowered productivity in the cod habitat, resulting in slower individual growth rates in cod (Dutil et al. 1999, 2003). The fast growth rate of cod under food-unlimited conditions (Björnsson and Steinarsson (2002) and the present study) may suggest that food availability (Björnsson 2001, 2002)

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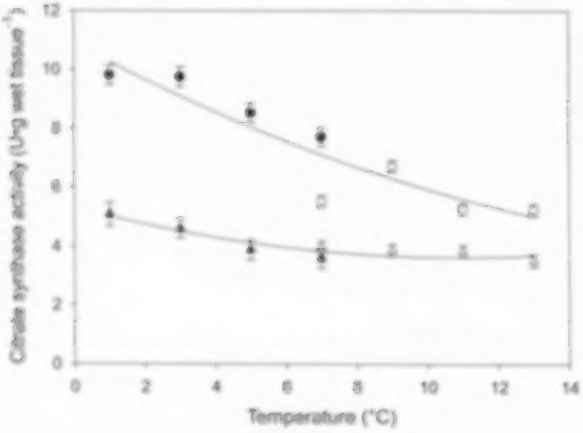
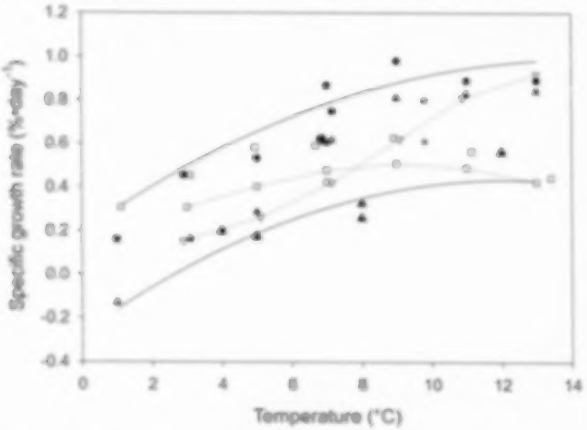
Fig. 5. Citrate synthase activity (least-square means and standard errors) in the intestine of Atlantic cod (*Gadus morhua*) from the southern Gulf of St. Lawrence (SG, circles) and the Bay of Fundy (BF, triangles) in relation to temperature in two experiments. Experiment 1 compared growth at lower temperatures (solid symbols; 1, 3, 5, 7 °C); experiment 2 compared growth at higher temperatures (open symbols; 7, 9, 11, 13 °C), respectively.



or changes in the diet and trophic relationships may explain some of the changes observed in lengths-at-age (Rose and O'Driscoll 2002; Sherwood et al. 2007; Fudge and Rose 2008).

Southern Gulf cod grow faster at low temperatures and perform better across a broader range of temperatures than Bay of Fundy cod. The two stocks inhabit different thermal habitats year round (ICES 2005), and southern Gulf cod live at a higher latitude and experience a shorter growth season than Bay of Fundy cod. Atlantic silversides (*Menidia menidia*) exhibited a latitudinal gradient in maximum growth potential across their range in the West Atlantic, with high-latitude fish growing faster (Conover and Present 1990). This was interpreted as resulting from size-dependent winter mortality, with high-latitude fish not being more adapted for growth at lower temperatures, but instead being able to grow more rapidly at higher temperatures during the shorter growth seasons that prevail at higher latitudes. In contrast, faster growth of southern Gulf compared with Bay of Fundy cod in the present study in cold water may reflect an adaptation to growth at low temperatures. Growth in length did not differ between stocks in the present study, and the high-latitude cold water stock did not outperform the low-latitude warm water stock at higher temperatures, possibly because food-unlimited growth rate of cod is maximum at intermediate temperatures (Björnsson and Steinarsson 2002; Björnsson et al. 2007). The warm water stock had low food efficiency below 7 °C and actually lost weight at the coldest temperature. Faster growth in transgenic coho salmon (*Oncorhynchus kisutch*) and Atlantic salmon (*Salmo salar*) was associated with an increased relative mass and increased surface area of the intestine (Stevens et al. 1999; Stevens and Devlin 2000). Size of the intestine did not increase at low temperatures in the present study, but citrate synthase activity of the intestine was enhanced. Higher specific citrate synthase activity in the intestine for southern Gulf cod at

Fig. 6. Compared specific growth rate (SGR) in mass of different stocks of Atlantic cod (*Gadus morhua*) fed to satiation at different temperatures. Observations for the southern Gulf of St. Lawrence and Bay of Fundy cod are shown as solid circles and solid triangles, respectively; smaller symbols are observations from the present study, larger symbols are from Lambert and Dutil (southern Gulf; Lambert and Dutil 2001), Kohler (Bay of Fundy; Kohler 1965), and Waiwood (Bay of Fundy; Waiwood 1978). Icelandic cod (Björnsson et al. 2001; Björnsson and Steinarsson 2002), open squares; Norwegian coastal cod (Hatlen et al. 2006, 2007), star. The dotted lines link values obtained from mathematical models: Icelandic cod (Björnsson et al. 2007), open squares; several European stocks (Jobling 1988), open triangles. The solid lines indicate approximate upper and lower boundaries for this relationship.



low temperatures further suggests that southern Gulf cod are better adapted for growth in cold water. Citrate synthase activity was enhanced in the pyloric caeca and intestine, and size of the intestine increased in cod fed ad libitum following a prolonged period of food deprivation (Blier et al. 2007). Increased capacity for energy production as indicated by citrate synthase activity may enhance active transport of nutrients across the gut wall at lower temperatures. Southern Gulf and Bay of Fundy cod also differ in the metabolic cost of maintenance, metabolic cost of transport, and sprint capacity, while maintaining comparable thermal sensitivities of metabolic capacities and swimming performance (Sylvestre et al. 2007).

The faster growth rate of southern Gulf cod at lower temperatures is consistent with the view that southern Gulf cod still have the capacity for fast growth, but some of the differences observed between stocks and between experiments in the present study may have resulted from uncontrolled factors. Although the initial length of the fish used in the experiments was the same, age was not determined and presumably differed between stocks. This would make our observations conservative because southern Gulf cod are, on average, 2–5 years older than Bay of Fundy cod of the same size for the range of sizes used in our study. Growth rate is expected to decrease with age, but this relationship may be driven by a correlation between age and length and by size effects on the metabolic and physiological processes. Size has a limiting effect on maximum growth rate, with slower growth rates being expected in heavier fish (Björnsson et al.

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2007). Based on a model of mass and temperature effects on specific growth rate for Icelandic cod (Björnsson et al. 2007), Bay of Fundy cod were expected to experience slower growth rates than southern Gulf cod, consistent with our results, but this size effect was much less than differences observed between the two stocks; the model predicts a 0.01% to 0.06% difference in specific growth rate in experiment 1 and a 0.01% to 0.02% difference in specific growth rate in experiment 2 due to mass differences alone. Much higher differences in specific growth rate were observed, particularly at low temperatures. Mass-at-length at the start of the experiments also differed between stocks, with southern Gulf cod having a lower condition factor than Bay of Fundy cod. This situation may have contributed to differences in growth rates observed during the experiments. Following a period of growth depression, fish may experience a burst of growth (Ali et al. 2003), a phenomenon known as compensatory growth (Bélanger et al. 2002; Dutil et al. 2006; Blier et al. 2007). Southern Gulf cod ate more food than Bay of Fundy cod at low temperatures. This is consistent with known mechanisms for compensatory growth. Although differences in condition factor between the two stocks when specimens were obtained from the wild may reflect a more favourable growth environment overall for the low-latitude mild-temperature stock, such differences cannot be interpreted as meaning that southern Gulf cod had experienced a period of growth depression in the few weeks before they were caught. Atlantic cod show a marked seasonal growth pattern, with growth taking place mainly in the summer period (Dutil and Lambert 2000; Mello and Rose 2005), but stocks differed in their average condition factor, with a more pronounced annual cycle in cold water stocks (fig. 9 in Dutil et al. 1999). The lack of difference in the specific growth rate between the two stocks at higher temperatures may suggest that compensatory growth explains differences observed in experiment 1; differences in condition factor were less in experiment 2. Furthermore, the difference in growth rate was less in experiment 2 than in experiment 1 at a common temperature (7 °C).

Food composition and seasonal timing of the experiments may have limited growth and may explain differences in growth rates between stocks and between experiments. Food composition may have had major effects on growth processes. The same diet was used in both experiments in the present study and included major prey items of cod (capelin and shrimp), but in contrast to other studies, a wet diet was used. Although a natural diet may shed some light on situations actually encountered by fish in their natural environments, natural diets may lead to an underestimate of maximum potential growth rates. Specific growth rate in mass correlated positively with protein content, whereas liver index correlated positively with lipid content of the diet in 200 g cod fed daily over a period of 28 weeks (Morais et al. 2001; Rosenlund et al. 2004). Voluntary food ingestion may also vary seasonally. Food intake was less in the spring experiment than in the fall experiment at low and high rations in cod reared at different salinities (Lambert et al. 1994). Food intake was less in the fall experiment than in the summer experiment in cod reared at different stocking densities, a situation that was ascribed to higher condition factors and the onset of maturation in the fall experiment (Lambert and Dutil 2001). There was only a minority of maturing fish in both experiments in the present study. This is explained by the size of fish selected for these experiments compared with the maturity curve for the two stocks (ICES 2005). Furthermore, southern Gulf cod spawn in spring (May–June), whereas Bay of Fundy cod (Western Scotian Shelf cod) spawn either in winter (February–March) or in the fall. This is consistent with a higher proportion of maturing fish in southern Gulf cod in experiment 1, which started in mid-November, and a higher proportion of maturing fish in Bay of Fundy cod in experiment 2, which started in mid-August. There was a corresponding difference in energy storage between the two experiments, with energy storage enhanced in late summer and early fall (experiment 2) and other processes being favoured later in fall and winter (experiment 1). In contrast, there were marked stock effects in protein accretion, but this situation was limited to experiment 1 at low ingestion rates and may reflect differences in the relationship between growth in mass and growth in length, as well as differences in condition at the start of the experiment between stocks in experiment 1. Condition effects were no longer important at high temperatures and maximum ingestion rates.

Somatic growth is a complex process influenced by numerous factors so that for fish of a similar size fed maximum rations and reared at similar temperatures, specific growth in length and mass varies. In the southern Gulf of St. Lawrence, cod sizes-at-age have declined in the 1990s; the stock has collapsed and has not recovered since then. The present study has shown that southern Gulf cod grow fast at low temperatures under food-unlimited conditions and possess physiological adaptations to life in cold water but perform as well as other stocks at higher temperatures. These observations are not consistent with alleged genetic consequences of the selective removal of fast-growing fish from that stock during long periods of high fishing mortalities in the precollapse period. They are, however, consistent with other hypotheses such as changes in prey availability and large-scale shifts in environmental conditions.

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Iteroparity in Columbia River summer-run steelhead (*Oncorhynchus mykiss*): implications for conservation

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Abstract: We used ultrasound imaging and passive integrated transponder (PIT)-tagging programs to assess maturation status and iteroparity patterns in summer-run steelhead (anadromous rainbow trout, *Oncorhynchus mykiss*) of the interior Columbia River Basin (Pacific Northwest, USA). Postspawn kelts examined in downstream fish bypass systems at Columbia River and Snake River dams were disproportionately female (>80%) and majorities were of wild origin, unlike prespawn steelhead at these sites. Annual repeat migration estimates varied from 2.9% to 9.0% for kelts tagged at lower Columbia River dams (n = 2542) and from 0.5% to 1.2% for Snake River kelts (n = 3762). Among-site differences reflected greater outmigration distance and additional dam passage hazards for Snake River kelts. There was also strong evidence for condition-dependent mortality, with returns an order of magnitude higher for good- versus poor-condition kelts. Disproportionately more females and wild fish also returned, providing potentially valuable genetic and demographic benefits for the Columbia River's threatened steelhead populations. Results overall provide baseline data for evaluating kelt mortality mitigation efforts and basic life history information for steelhead conservation planning.

Résumé : L'imagerie ultrasonique et les programmes de marquage transpondeurs intégrés passifs (PIT) nous servent à évaluer le statut de maturation et les patrons d'itéroparité chez les truites arc-en-ciel anadromes (*Oncorhynchus mykiss*) à montaison estivale du bassin intérieur du Columbia (nord-ouest pacifique, É.-U.). Les charognards d'après la fraie examinés dans les systèmes de passes migratoires de dérivation vers l'aval aux barrages des rivières Columbia et Snake sont de façon disproportionnée de sexe féminin (>80 %) et la plupart des individus sont d'origine sauvage, contrairement aux truites arc-en-ciel anadromes à ces mêmes sites avant la fraie. Les estimations de l'importance des migrations annuelles répétées varient de 2,9–9,0 % chez les charognards marqués aux barrages du Columbia inférieur (n = 2542) et de 0,5–1,2 % chez les charognards de la Snake (n = 3762). Les différences entre les sites reflètent la plus grande distance de migration vers la mer et les risques additionnels du passage des barrages pour les charognards de la Snake. Il y a aussi de fortes indications de l'existence d'une mortalité reliée à la condition; en effet, les retours sont dix fois plus abondants chez les charognards en bonne condition que chez ceux en mauvaise condition. De façon disproportionnée, il y a aussi plus de retours de femelles et de poissons sauvages, ce qui apporte potentiellement des bénéfices génétiques et démographiques précieux aux populations menacées de truites arc-en-ciel anadromes du Columbia. Globalement, nos résultats fournissent les données de base nécessaires pour évaluer les efforts de mitigation de la mortalité des charognards, ainsi que des informations démographiques fondamentales pour planifier la conservation de la truite arc-en-ciel anadrome.

[Traduit par la Rédaction]

Introduction

Postspawning survival rates vary widely among the diadromous Salmoninae, from relatively high survival for *Salvelinus* and some *Salmo* species to complete semelparity (death following first spawning) in most but not all *Oncorhynchus* species (Rounsefell 1958; Fleming 1998). Semelparous and iteroparous (repeat spawning) life history strategies share a common evolutionary lineage, with semelparity believed to have developed last in response to several interrelated selective pressures (Stearley 1992). These include increased energetic costs associated with anadromy, long-distance migration, and breeding competition and also greater reproductive investments in secondary sexual characteristics, body size, and egg size (Willson 1997; Crespi and Teo 2002). In contrast, iteroparous life history types allocate relatively less energy to each reproductive episode and relatively more to postspawning survival (Dodson 1997). This

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strategy spreads the risk associated with catastrophic reproductive failure by allowing multiple spawning events by individual fish (Fleming and Reynolds 2004; Wilbur and Rudolf 2006).

Iteroparity, like variable age at maturity (Groot and Margolis 1991), may offer several population-level advantages. Both strategies maintain genetic diversity and reduce demographic risks by increasing the number of unique adult pairings across years (Crespi and Teo 2002; Niemelä et al. 2006). Iteroparous individuals may also be more productive than semelparous conspecifics because of higher cumulative fecundity and lifetime fitness (Fleming and Reynolds 2004). This may be especially important for females, given their strong influence on nest site selection, spawn timing, and early juvenile survival (Fleming 1996; Quinn 2005). Iteroparity within populations is typically dominated by females (Withler 1966; Fleming 1998), improving sex ratios that are often strongly skewed towards males (Mills 1989; Burgner et al. 1992; Willson 1997).

The benefits of iteroparity are unrealized in many anadromous populations because human activities typically select against repeat spawning by increasing adult and kelt (post-spawned adult) mortality (Altukhov et al. 2000; Crespi and Teo 2002). For example, harvest rates often differ among maiden and repeat spawners because of size-selective fisheries, migration timing differences, and (or) ocean distribution patterns (e.g., Dempson et al. 2004). Additionally, hydroelectric dams and other fish passage barriers present a variety of direct and indirect mortality hazards for outmigrating kelts (Wertheimer and Evans 2005; Arnekleiv et al. 2007; Scruton et al. 2007) that differ from those for upstream migrants. Kelts are often emaciated, with limited somatic energy reserves and reduced swimming abilities (Booth et al. 1997; Scruton et al. 2007), and consequently they are especially vulnerable to entrainment in hydroelectric turbines or other hazardous passage routes. Further, dams and reservoirs can slow kelt outmigrations by reducing water velocities and increasing time spent searching for passage routes (e.g., Wertheimer and Evans 2005; Wertheimer 2007). These delays have direct energetic costs and postpone the critical resumption of ocean feeding and gonadal recrudescence.

The outmigration environment for steelhead (anadromous rainbow trout, *Oncorhynchus mykiss*) kelts in the Columbia River Basin is one of the more difficult among iteroparous populations. In addition to long downstream migration distances (up to ~1500 km), summer-run (freshwater maturing) kelts must pass as many as nine hydroelectric dams and reservoirs to reach the Pacific Ocean. Kelt migration mortality in the impounded portion of this system can be very high (>95% in some years, Wertheimer and Evans 2005), suggesting strong selection against iteroparous forms. Recent documentation of this mortality has focused attention on improving kelt survival in the Columbia system, where broad-based population declines have resulted in US Endangered Species Act listing of most interior steelhead stocks (National Marine Fisheries Service 1997; Good et al. 2005). Current steelhead recovery efforts include increasing iteroparity to take advantage of genetic and demographic benefits of repeat spawners (National Marine Fisheries Service 2000).

Although the Columbia River historically supported some of the largest and most diverse steelhead runs in North America (Brannon et al. 2004; Augerot 2005), little is known about historic or current iteroparity patterns. A single predam publication reported repeat spawner rates of 2%, 4%, and 12% for summer, fall (freshwater maturing), and winter (ocean maturing) runs, respectively (Long and Griffin 1937). These estimates were based on scale samples collected at multiple but unspecified sites. More recent estimates have mostly been ≤5% for a small number of interior summer steelhead stocks and up to 17% for some winter-run populations returning to sites downstream from all dams (Whitt 1954; Leider et al. 1986; Meehan and Bjornn 1991).

In this study, we addressed some of the basic iteroparity information gaps in the aggregated summer-run steelhead population of the interior Columbia River Basin. There were three interrelated study objectives. First, we collected kelt demographic and condition data at collection sites along the outmigration corridor, including fish sex, size, origin (hatchery versus wild), and coloration. Second, we examined associations among repeat spawner return rates and kelt demographics, outmigration timing, collection location, and year. We used an information-theoretic approach to assess the relative roles of these factors in predicting repeat spawner returns because the collected data were observational. Third, we examined life history characteristics of returning fish, including breeding interval, migration timing, and distribution within the Columbia River Basin. The combined objectives tested the following hypotheses: (*i*) repeat spawner return rates would be affected by outmigration distance, with lower returns expected for kelts collected at sites more distant from the ocean; (*ii*) return rates would differ among demographic groups, with higher returns expected for females, wild fish, and kelts in good physical condition; (*iii*) return rates would differ with outmigration timing, with higher returns expected for fish outmigrating during the peak of the kelt runs when rapidly increasing snowmelt runoff would presumably increase migration speed; and (*iv*) breeding interval would differ with outmigration timing and distance given their potential effects on ocean entry timing. We expected that some late-timed kelts and those with the longest migrations would enter the ocean with insufficient time to restore lost energy reserves and remature within a single season.

Materials and methods

Fish collection and tagging

Adult steelhead (both kelts and prespawners that were falling back downstream; Boggs et al. 2004) were collected from juvenile bypass systems at John Day and McNary dams on the Columbia River and at Lower Granite Dam on the Snake River (Fig. 1). These sites were 347, 470, and 695 river kilometres (rkm) from the Pacific Ocean, respectively. Sampling occurred from mid-March or early April through late May or mid-June in 4 years at John Day Dam (2001–2004) and 3 years each at McNary (2001–2002, 2004) and Lower Granite (2002–2004) dams (Fig. 2). In total, maturation status was evaluated for 13 193 adult steelhead: 4394 at John Day, 1390 at McNary, and 7409 at Lower Granite. Fish were directly diverted (John Day) or transferred via

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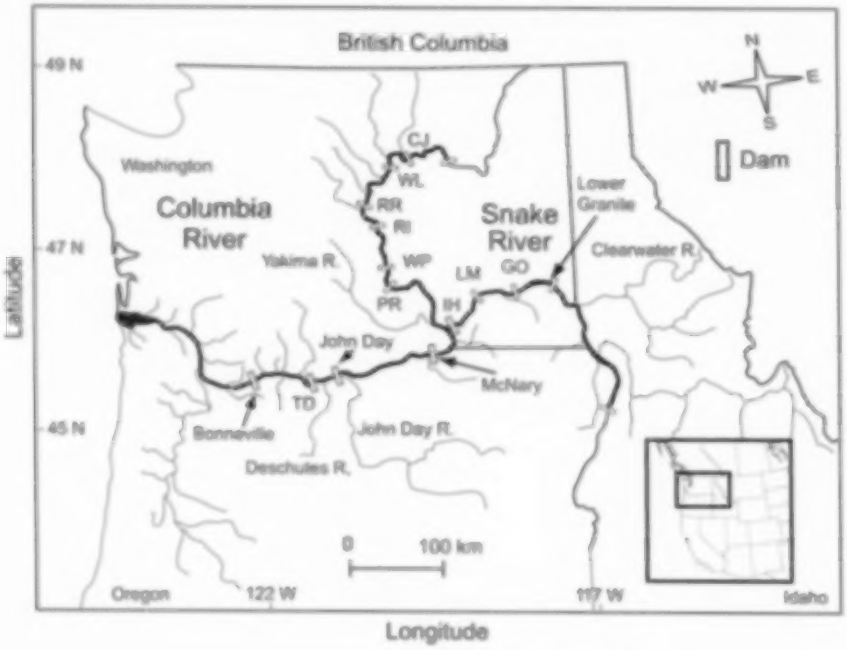
Fig. 1. Map of the Columbia and Snake rivers showing mainstem dams and major tributaries mentioned in the text; inset shows locations in the Pacific Northwest, USA. Steelhead (*Oncorhynchus mykiss*) kelts were collected at John Day, McNary, and Lower Granite dams in 2001–2004. Repeat spawners were detected at passive integrated transponder (PIT) tag interrogators at Bonneville, McNary, Ice Harbor (IH), Lower Granite, Priest Rapids (PR), Rock Island (RI), and (or) Wells (WL) dams in 2001–2005. Other dams include Wanapum (WP), Rocky Reach (RR), Chief Joseph (CJ), Lower Monumental (LM), and Little Goose (GO).



dip net or flumes (McNary, Lower Granite) to nearby tanks containing aerated river water and anesthetic (60 ppm tricaine methanesulfonate (MS-222) or 30 mg·L^{-1} of clove oil) (Prince and Powell 2000; Pirhonen and Schreck 2003). While fish were anesthetized, we recorded fork length (cm), overall physical condition (rated as good, fair, poor), coloration (rated as bright, intermediate, dark), origin (hatchery, wild), and sex. Condition was based on the degree of visible external damage (e.g., abrasions, lesions, fungal infections; see Evans 2003), while coloration (an indicator of physiological state) was based on the degree of the fish's silvery, ocean-like external appearance. Clipped adipose fins indicated hatchery origin and fish with adipose fins were presumed wild unless other hatchery marks were evident (e.g., blunt dorsal fins). Ultrasound imaging of gonads was used to identify sex and distinguish prespawn steelhead from postspawn kelts using the technique described in Evans et al. (2004*b*).

Uniquely coded passive integrated transponder (PIT) tags were injected into the pelvic girdle of 52%–55% of the kelts sampled at each dam (n = 1946 at John Day; 596 at McNary; 3762 at Lower Granite). PIT tags were used because retention rates are high, tag life is unrestricted, and negative tag effects are minimal (Gibbons and Andrews 2004). PIT-tagging percentages varied across weeks and years and among dams (Fig. 2) as a result of personnel availability and evolving study objectives. At all sites, kelts selected for PIT tagging were more likely than the full kelt samples to be in good or fair condition (Table 1), resulting in higher percentages of tagged wild and female kelts compared with the full samples. These tagging patterns added some potential bias to return evaluations in that brighter, good-condition kelts were disproportionately tagged. However, kelts of all condition types, sexes, and origins were tagged at all sites in all years.

Many PIT-tagged kelts (6%–38%) were also externally radio-tagged (see Wertheimer and Evans 2005 for summary data). Telemetry studies were in 2001, 2002, and 2004 at John Day and McNary dams and in 2002 and 2003 at Lower Granite Dam. Following tagging, all kelts recovered in temporary holding tanks until equilibrium was regained; fish then volitionally exited or were transferred directly into the dam's tailrace to continue downstream migration.

Repeat spawner monitoring

Steelhead returning on repeat spawning migrations could be enumerated at a series of PIT tag interrogation systems in dam fish ladders (e.g., Keefer et al. 2008*b*). PIT detection systems were in place at Bonneville and Lower Granite dams in 2001 and were added at McNary and Wells dams in 2002 and at Priest Rapids, Rock Island, and Ice Harbor dams in 2003. Detection efficiency estimates for the PIT tag systems were >90% for adult steelhead in independent evaluations at Bonneville and McNary dams in 2001 and 2002 (Downing and Prentice 2003), and efficiencies improved as a result of system refinements and interrogator redundancy in subsequent years. The probability of a repeat-spawn steelhead passing upstream undetected was therefore

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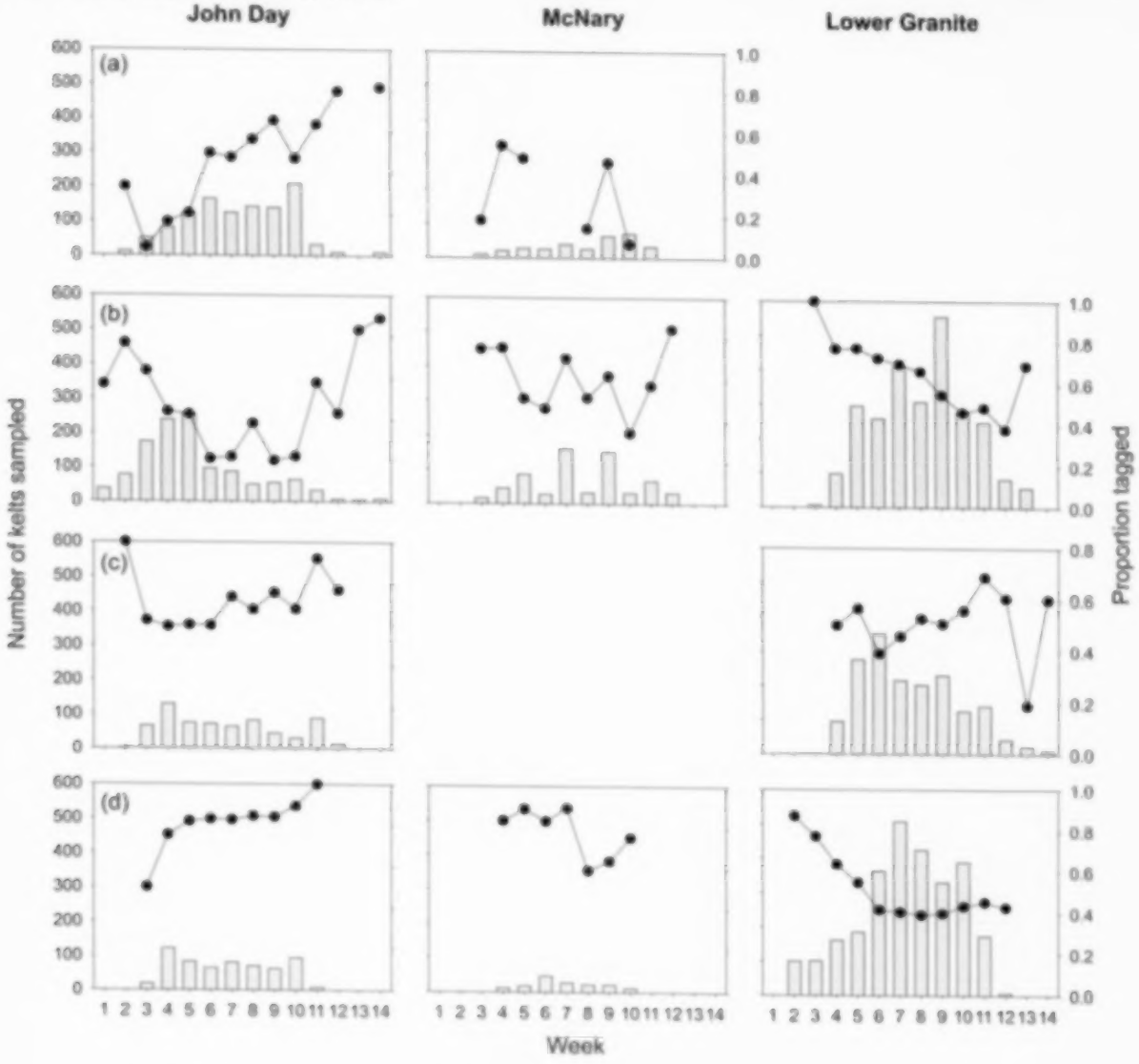

Fig. 2. Numbers of steelhead (*Oncorhynchus mykiss*) kelts sampled at John Day, McNary, and Lower Granite dams (bars) in 2001–2004, with the proportions that were passive integrated transponder (PIT)-tagged (●): rows (*a*) = 2001; (*b*) = 2002; (*c*) = 2003; (*d*) = 2004. Week 1 = 18–24 March; week 14 = 17–23 June.

both low and increasingly unlikely as the kelt studies progressed. In addition, steelhead from upriver spawning populations were less likely to pass undetected, as their route included multiple interrogator sites. Repeat spawner rates presented here, however, are still minimums, as PIT tag retention rates (i.e., proportion of tagged fish that retained tags) and the effects of kelt handling or tagging on survival were not quantified; these effects were considered to be minimal sources of bias.

Statistical analyses

The likelihood of steelhead returning on repeat spawning migrations was examined using a combination of univariate analyses (e.g., Pearson χ^2 and Fisher's exact tests) and multiple logistic regression models. Predictor variables included kelt characteristics (condition, coloration, length, sex, origin) and (or) outmigration metrics (year, release week, release site). These terms were not strictly independent (e.g., kelts in good condition were more likely to be brightly colored and female). A preliminary logistic regression model included all PIT-tagged kelts from all sites and years to assess differences among release locations and gross differences related to fish characteristics. A series of additional logistic models were run separately for each release site and were limited to fish in good condition in an effort to minimize the possible effects of tagging bias due to dispro-

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Table 1. Origin, sex, and condition summaries for all steelhead (*Oncorhynchus mykiss*) kelts sampled annually from 2001 to 2004, including the total subsamples of passive integrated transponder (PIT)-tagged fish.

			Origin (%)		Sex (%)*			Condition (%)			
Dam	Year	*n*	Wild	Hatchery	F	M	U	Dead	Poor	Fair	Good
John Day	2001	1096	76	24	57	5	39	2	26	24	48
	2002	1188	58	42	64	12	24	4	47	23	27
	2003	669	73	27	69	9	22	5	45	22	28
	2004	607	73	27	61	17	23	4	34	24	39
	Total	3560	69	31	62	10	28	3	38	23	36
	PIT	1946	71	29	63	11	27	—	25	27	47
McNary	2001	330	63	37	53	5	42	1	32	14	53
	2002	650	66	34	74	11	15	2	40	15	43
	2004	161	70	30	70	15	15	2	27	24	47
	Total	1141	66	34	67	10	23	2	36	16	46
	PIT	596	71	29	73	11	17	—	11	23	66
Lower Granite	2002	2610	51	50	83	17	—	—	24	28	48
	2003	1714	50	50	83	17	—	—	27	26	46
	2004	2744	56	45	84	16	—	—	18	42	40
	Total	7068	52	48	83	17	—	—	22	33	44
	PIT	3762	53	47	84	16	—	—	11	38	51

*F, female; M, male; U, unknown.

portionate sampling. We compared these models using information-theoretic techniques (Burnham and Anderson 2002). The candidate models included univariate terms, several a priori subsets of the predictor variables (i.e., fish characteristics), and the most parsimonious subsets identified using backward stepwise regression (Hosmer and Lemeshow 2000). All of the main effects models were ranked using Akaike's information criterion (AIC) and evaluated with respect to ΔAIC, the change in AIC relative to the best or most parsimonious model (Buckland et al. 1997; Burnham and Anderson 2002). Interaction terms were not included in the AIC model selection procedure. However, associations between all first-order interactions and kelt return rates were evaluated in separate models to assess their influence relative to individual predictor variables.

Varying proportions of repeat spawners returned in the same calendar year as their outmigration (hereafter referred to as consecutive repeat spawners) or returned the calendar year after their outmigration (hereafter referred to as skip repeat spawners because of the additional winter at sea). Consecutive and skip return percentages were compared among kelt release sites and among years using χ^2 tests. Outmigration and return migration timing distributions for these groups were compared using Kruskal–Wallis tests, and analysis of variance was used to assess fork length differences among groups.

Results

Maturation status

Across years, 81%–82% of John Day and McNary samples and 95% of the Lower Granite sample were determined to be kelts, 17%–18% were considered prespawners, and maturation status was undetermined for 1% of all fish. Prespawners were more numerous in late March to early April, when they made up 30%–60% of samples at all sites. Thereafter, percentages of kelts rapidly increased and made up >90% of almost all mean weekly samples in May and June. Prespawners were more likely than kelts to be of hatchery origin at John Day (58% of 828 prespawners versus 31% of 3560 kelts) and Lower Granite (61% of 317 prespawners versus 48% of 7068 kelts) dams, but not at McNary Dam (31% of 248 prespawners versus 34% of 1141 kelts).

Kelt samples

Kelt characteristics (i.e., origin, sex, condition, length, and coloration) varied across sites and years and within year. Overall, 66%–69% of all kelts sampled at John Day and McNary dams and 52% at Lower Granite Dam were wild origin (Table 1). Percent wild differed significantly among dams each year ($\chi^2 \geq 24.6$, $P < 0.001$). Of those kelts that could be confidently sexed, far more were female (83%–87%) than male (13%–17%) at all sites in all years. Sex assignment was unknown for about a quarter of the kelts evaluated at John Day and McNary dams, primarily because gonads had been completely evacuated or absorbed following spawning, leaving no discernable eggs or testes (Table 1). At John Day Dam, unknown sex fish were morphologically more similar to females than males on average (e.g., tended to be larger, brighter, and in better condition); patterns were equivocal for unsexed fish at McNary Dam. With all years combined and unknown sex fish excluded, sex ratios did not differ by kelt origin (hatchery, wild) at John Day or Lower Granite dams ($P \geq 0.13$), while at McNary Dam, 66% of females were wild versus 79% of males ($\chi^2 = 7.0$, $P = 0.008$). In individual years, significantly ($P < 0.05$) more males than females were wild at Lower Granite and McNary dams in 2002; the opposite was true at Lower Granite Dam in 2003.

Kelt condition (good, fair, poor) was better overall for fish at Lower Granite (44% good condition) and McNary

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Table 2. Results of multiple logistic regression model to predict repeat spawner returns of steelhead (*Oncorhynchus mykiss*) kelts using all fish passive integrated transponder (PIT)-tagged in all years.

Effect	df	χ^2	*P*	Effect	Odds ratio	95% CI
Tag site	2	48.79	<0.001	John Day vs. Lower Granite	5.88	3.73–9.67
				McNary vs. Lower Granite	3.94	2.23–6.97
Condition	2	31.95	<0.001	Good vs. poor	25.36	6.10–105.43
				Fair vs. poor	10.27	2.40–43.84
Week	1	20.30	<0.001	—	0.85	0.79–0.91
Coloration	2	9.85	0.007	Dark vs. bright	0.22	0.05–0.97
				Intermediate vs. bright	0.60	0.42–0.87
Origin	1	8.19	0.004	Hatchery vs. wild	0.56	0.37–0.83
Year	3	7.73	0.052	2001 vs. 2004	1.10	0.69–1.76
				2002 vs. 2004	1.14	0.75–1.74
				2003 vs. 2004	0.51	0.22–1.13
Sex	2	3.27	0.195	Male vs. female	0.65	0.30–1.39
				Unknown vs. female	1.29	0.87–1.90
Length	1	1.75	0.186	—	0.98	0.96–1.01

Note: Predictor variables included collection location, year, and week and kelt condition, color, origin, sex, and fork length. River discharge and dam spill terms were excluded because these variables varied across sites. No fish were PIT-tagged at Lower Granite Dam in 2001 or McNary Dam in 2003, but a reduced model that included data from 2002 and 2004 only produced qualitatively similar results. CI, confidence interval.

(46% good condition) dams than at John Day Dam (36% good condition) (Table 1). Condition differed among sites in each year and with all years combined ($\chi^2 \geq 15.4$, $P < 0.001$). Wild kelts were in better overall condition than hatchery kelts at each site ($\chi^2 \geq 21.1$, $P < 0.001$, all years combined), and more females than males were in good condition at each site ($\chi^2 \geq 16.0$, $P < 0.001$). These sex- and origin-related condition differences were also significant ($P < 0.05$) in most individual site × year samples. Kelt fork length also differed ($P \leq 0.05$, *t* tests) among sites and by origin and sex. On average, the largest kelts were at Lower Granite Dam, females were larger than males, and wild fish were larger than hatchery fish. Although significant, differences in means were generally ≤4 cm.

Kelt origin varied within season in several ways. Percentages of wild kelts increased with increasing migration date at John Day and McNary dams, from ~60% early in the migrations to ~80% during the last sampling weeks. At Lower Granite Dam, the mean wild kelt percentage decreased from ~60% early in the migration to ~30% in midseason and then rapidly increased to nearly 100% by late migration in all years. Sex ratios were relatively constant through time at all sites, with females outnumbering males. Percentages of kelts in good condition and with bright coloration tended to increase within season, while dark kelts and kelts in poor condition decreased at all sites.

Return rates

Across release sites and years, 164 of 6304 (2.60%) PIT-tagged kelts were detected on repeat spawning migrations, and two of these (0.03%) were recorded returning twice (i.e., on a third migration). A full-sample multiple logistic regression model that included release site, migration timing, and kelt characteristic variables indicated that release site, kelt condition, and release week were the most influential predictors of returns (Table 2). Odds ratios showed kelts tagged at John Day Dam and McNary Dam were 5.9 and 3.9 times more likely to return than those tagged at Lower Granite Dam, respectively. Kelts in good or fair condition were respectively >25 and >10 times more likely to return than those in poor condition. Early-timed, bright colored, and wild origin kelts were also significantly ($P < 0.05$) more likely to return. There was also evidence for year effects ($P = 0.052$, Table 2), while fish length and sex were nonsignificant terms in the full model.

Although sex was not included in the full logistic regression model, proportionately more females than males returned from all release sites (2.30% versus 1.22%), and females returned at higher rates than males at all sites in all years (Table 3). These differences were not significant in univariate tests with all years combined or for any individual site × year sample ($P \geq 0.10$; male versus female only, Table 3). The presence of unsexed fish at two of three study sites, however, may have affected this nonsignificant finding, especially given that unsexed fish returned at the highest rates. When sex data was limited to kelts tagged at Lower Granite Dam (where 100% of kelts were classified as male or female), a higher percentage of females returned as repeat spawners (0.79%, 25 fish) relative to males (0.17%, 1 fish). The small numbers of returning fish from both sexes, however, limited statistical comparisons.

Other univariate evaluations were also informative. Across years, return percentages were 5.45% (106/1946) for John Day releases, 5.37% (32/596) for McNary releases, and 0.69% (26/3762) for Lower Granite releases (Table 3). Among-site differences were significant with all years combined ($\chi^2 = 134.39$, $P < 0.001$) and in each year that fish were tagged at Lower Granite Dam ($\chi^2 \geq 15.94$, $P < 0.001$). Among-year return differences were significant for John Day ($\chi^2 = 11.14$, $P = 0.011$) and Lower Granite ($\chi^2 = 6.77$, $P = 0.034$) releases, with the lowest returns in 2003 for both sites (Table 3). Kelts in good condition returned at higher rates (9.22%, John Day; 7.16%, McNary; 1.19%, Lower Granite) than those in poor condition (0.20%, John Day; 1.54%, McNary; 0.00%, Lower Granite) for all release sites ($P \leq 0.027$, all years combined) (Table 3). Condition effects

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Table 3. Summary of steelhead (*Oncorhynchus mykiss*) return rates (%) by kelt passive integrated transponder (PIT)-tagging site, outmigration year, sex, origin, and condition.

		Year				
Dam	Variable	2001	2002	2003	2004	Total
John Day	Female	7.72 (298)	5.59 (322)	2.32 (302)	4.00 (300)*	4.91 (1222)*†
	Male	0.00 (15)	4.11 (73)	0.00 (43)	2.63 (76)*	2.42 (207)*
	Unknown	8.72 (172)	5.60 (125)	5.66 (106)	11.40 (114)*	7.93 (517)*
	Wild	9.07 (386)*	5.37 (298)	3.57 (336)	5.43 (368)	5.98 (1388)†
	Hatchery	3.03 (99)*	5.41 (222)	0.87 (115)	5.74 (122)	4.12 (558)
	Poor	0.00 (10)	0.42 (240)*	0.00 (124)*	0.00 (115)*	0.20 (489)*
	Fair	5.94 (101)	6.04 (149)*	1.42 (141)*	2.08 (144)*	3.74 (535)*
	Good	8.56 (374)	13.74 (131)*	5.91 (186)*	10.39 (231)*	9.22 (922)*
	Total	7.84 (485)	5.38 (520)	2.88 (451)	5.51 (490)	5.45 (1946)†
McNary	Female	5.56 (36)	6.13 (310)	—	5.75 (87)	6.00 (433)
	Male	0.00 (2)	4.88 (41)	—	0.00 (20)	3.17 (63)
	Unknown	13.79 (29)	0.00 (52)	—	0.00 (19)	4.00 (100)†
	Wild	7.55 (53)	6.47 (278)	—	5.43 (92)	6.38 (423)
	Hatchery	14.29 (14)	2.40 (125)	—	0.00 (34)	2.89 (173)†
	Poor	0.00 (2)	2.00 (50)*	—	0.00 (13)	1.54 (65)*
	Fair	7.69 (13)	1.11 (90)*	—	2.70 (37)	2.14 (140)*
	Good	9.62 (52)	7.22 (263)*	—	5.26 (76)	7.16 (391)*
	Total	8.96 (67)	5.21 (403)	—	3.97 (126)	5.37 (596)
Lower Granite	Female	—	0.59 (1350)	0.41 (740)	1.29 (1083)	0.79 (3173)
	Male	—	0.00 (267)	0.00 (128)	0.52 (194)	0.17 (589)
	Wild	—	0.51 (780)	0.62 (483)	1.64 (732)	0.95 (1995)*
	Hatchery	—	0.48 (837)	0.00 (385)	0.55 (545)	0.40 (1767)*
	Poor	—	0.00 (402)*	0.00 (4)	0.00 (5)	0.00 (411)*
	Fair	—	0.00 (446)*	0.00 (336)	0.47 (637)	0.21 (1419)*
	Good	—	1.04 (769)*	0.57 (528)	1.89 (635)	1.19 (1932)*
	Total	—	0.49 (1617)	0.35 (868)	1.17 (1277)	0.69 (3762)†

*Varied ($P < 0.05$) across category (i.e., sex, origin, condition).
†Varied ($P < 0.05$) across years.

were consistent for individual site × year samples (Table 3) and across most combinations of sex and origin. Wild kelts returned at higher rates than hatchery kelts from all release sites (5.98% versus 4.12%, John Day; 6.38% versus 2.89%, McNary; 0.95% versus 0.40%, Lower Granite; Table 3). This pattern was significant for the Lower Granite sample with all years combined ($\chi^2 = 4.22$, $P = 0.040$) and the John Day sample in 2001 ($\chi^2 = 3.98$, $P = 0.046$).

In comparisons of models using kelt characteristics, migration year, and migration timing variables, outmigration week was the most informative univariate predictor for good-condition fish at all release sites (Table 4). Later migrants were less likely to return in all cases, with odds ratios of 0.89 (95% CI = 0.81–0.97; $\chi^2 = 7.03$, $P = 0.008$) for John Day, 0.84 (95% CI = 0.70–0.99; $\chi^2 = 3.88$, $P = 0.049$) for McNary, and 0.75 (95% CI = 0.63–0.90; $\chi^2 = 9.93$, $P = 0.002$) for Lower Granite releases.

Among the a priori models, the model with all six terms was the most parsimonious for John Day releases (Table 4). The year + week model was best for McNary and Lower Granite kelts. The model identified using backward stepwise selection was different for each release site (Table 4). The stepwise model for the John Day sample (week + length + color) indicated the kelts most likely to return were early migrating ($\chi^2 = 8.97$, $P = 0.003$), bright colored ($\chi^2 = 8.76$, $P = 0.013$), and relatively smaller ($\chi^2 = 3.72$, $P = 0.054$). The McNary model (week) indicated returns were highest for early outmigrants ($\chi^2 = 3.88$, $P = 0.049$), and the Lower Granite model (week + origin) indicated wild ($\chi^2 = 3.88$, $P = 0.049$), early migrants ($\chi^2 = 10.94$, $P < 0.001$) were most likely to return (Table 4).

Generally, the 15 first-order interaction terms explained little additional variation than the main effects models or the stepwise regression results. For all three release sites, the lowest AIC values were for interaction terms that included week (e.g., week × length, week × color), suggesting an overall outmigration timing effect. AIC values for these terms were similar to or slightly higher than those for the univariate and a priori models in Table 4.

There was no evidence that radio tagging negatively affected returns relative to PIT tagging. Return percentage comparisons based on the two tagging procedures showed no significant ($P > 0.05$) differences at any site or in pairwise tests based on site and either condition, origin, or sex. We note that none of 347 radio-tagged kelts from Lower Granite returned (versus 0.69% for the full sample).

Breeding interval, migration timing, and distribution of repeat spawners

Of 164 PIT-tagged kelts that returned as repeat spawners,

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Table 4. Logistic regression model selection and comparison statistics used to predict if steelhead (*Oncorhynchus mykiss*) kelts in good condition returned to spawn, by collection site.

		John Day		McNary		Lower Granite	
Model	df	AIC	ΔAIC	AIC	ΔAIC	AIC	ΔAIC
Univariate							
Year	2–3	569.0	11.2	206.7	5.2	251.0	10.2
Week	1	564.0	6.1	201.5	0.0	243.1	2.3
Origin	1	570.2	12.4	203.2	1.7	250.2	9.4
Sex	1–2	570.1	12.2	205.7	4.2	251.4	10.6
Length	1	569.6	11.7	204.3	2.8	253.4	12.6
Color	1–2	566.0	8.1	NA*	NA*	253.7	12.9
A priori							
Year + week	3–4	565.9	8.1	204.4	2.9	244.5	3.7
Origin + sex	2–3	571.0	13.2	205.3	3.8	250.0	9.2
Sex + length	2–3	570.4	12.6	206.8	5.3	253.0	12.2
Origin + sex + length + color	5–6	567.3	9.5	NA*	NA*	253.7	12.9
Year + week + origin + sex + length + color	8–10	561.5	3.7	NA*	NA*	246.1	5.3
Backward stepwise selection							
Week + length + color	4	557.8	0.0	—	—	—	—
Week	1	—	—	201.5	0.0	—	—
Week + origin	2	—	—	—	—	240.8	0.0

Note: Models include each univariate predictor, several a priori models, and models identified using backward stepwise selection ($P < 0.1$ as the cutoff for variable retention).

*No "dark" kelts returned from McNary Dam releases.

57% ($n = 94$) were consecutive repeat spawners, 43% ($n = 70$) were skip repeat spawners, and at least one fish exhibited both return strategies (i.e., returned twice). Kelts tagged at John Day Dam were more likely to be consecutive spawners (65%) than those tagged at McNary (47%) or Lower Granite (38%) ($\chi^2 = 7.83$, $P = 0.020$) dams. Generally, breeding interval (consecutive, skip) did not differ by kelt origin or condition within release site ($P > 0.05$). For McNary fish, consecutive return percentages differed by year (2001 > 2004 > 2002; $\chi^2 = 9.97$; $P = 0.007$). Consecutive spawners tended to have longer fork length than skip spawners (2.1–2.7 cm, on average across years), but differences were significant ($P < 0.05$, analysis of variance (ANOVA)) only for the 2002 John Day and 2004 McNary samples. Twelve skip spawners were recaptured at Bonneville Dam on return migrations in 2003; mean fork lengths for these fish were 57 cm (range = 52–62 cm) when they were PIT-tagged as kelts and 69 cm (range = 63–77) upon recapture.

Consecutive spawners outmigrated earlier as kelts than skip spawners (Fig. 3), with median outmigration dates earlier by 11 days (John Day releases), 7 days (McNary releases), and 16 days (Lower Granite releases) with all years combined ($P \leq 0.037$, Kruskal–Wallis tests). This pattern was consistent in all years for all release sites but was generally not significant ($P > 0.05$). When evaluated within a given return year, return migration timing also differed between consecutive and skip spawners (Fig. 4). Median return dates at Bonneville Dam for consecutive returns were 33 days (John Day releases), 45 days (McNary releases), and 28 days (Lower Granite releases) later than for skip returns. With all years combined, these differences were significant ($P < 0.001$, Kruskal–Wallis tests) for fish tagged at John Day and McNary dams but not at Lower Granite Dam ($P = 0.079$). Patterns were similar for the majority of individual site × year samples, but were generally not significant ($P > 0.05$).

Overall, 97% of known returning steelhead were detected at Bonneville Dam PIT tag interrogators, and percentages were consistently high across kelt release sites and years. On average, 36% of returning fish from the John Day tagging group were detected at McNary Dam, 8%–11% were detected at Snake River dams, and 4%–7% were detected at mid-Columbia River dams. Means for the McNary release group were 97% at McNary Dam, 20%–23% at Snake River dams, and 5%–26% at mid-Columbia River dams. These patterns suggest that many kelts tagged at John Day Dam originated in the John Day River, while many kelts tagged at both John Day and McNary dams were from sites other than the Snake River. In all cases, additional fish almost certainly returned to mid-Columbia sites, where PIT monitoring did not begin until 2002 (Wells Dam) or 2003 (Priest Rapids and Rock Island dams).

Discussion

Iteroparity patterns

Steelhead return rates in the Columbia River Basin varied along several gradients that mostly supported our hypotheses. First, there was a clear negative relationship between kelt outmigration distance and repeat spawner return rates, with kelts tagged at John Day Dam (third mainstem dam, rkm 347) about six times more likely to return than those tagged at Lower Granite Dam (eighth mainstem dam, rkm 695). This likely reflects a combination of life history differences among populations and some level of distance- and (or) dam-related migration mortality for upriver kelts. Second, there was persuasive evidence for condition-dependent

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Fig. 3. Outmigration timing distributions of steelhead (*Oncorhynchus mykiss*) kelts that were consecutive spawners (shaded boxes) or skip spawners (open boxes) by kelt collection site. Boxes show median, quartile, and 5th and 95th percentiles.

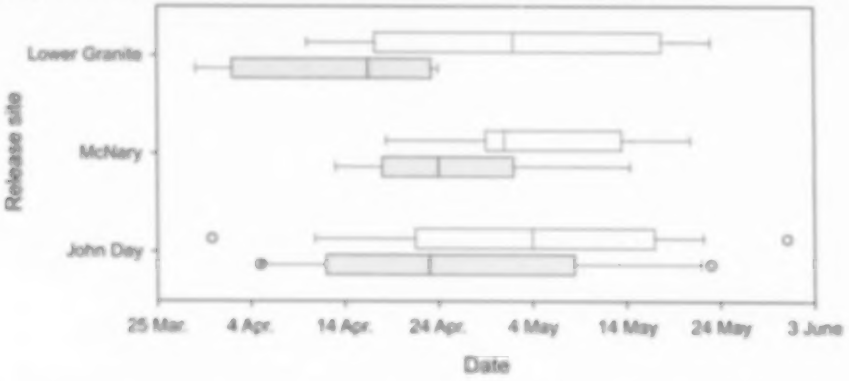
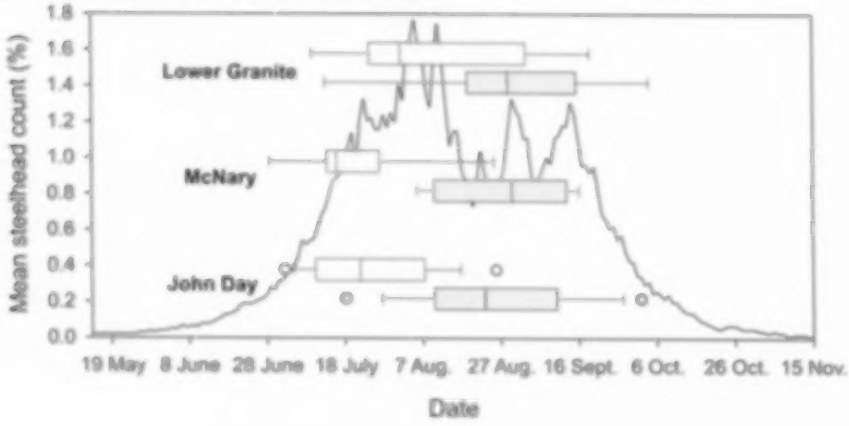


Fig. 4. Return timing distributions at Bonneville Dam for steelhead (*Oncorhynchus mykiss*) that were consecutive spawners (shaded boxes) or skip spawners (open boxes) by kelt collection site. Boxes show median, quartile, and 5th and 95th percentiles. Line shows the mean steelhead count (expressed as %) at Bonneville Dam from 2001 to 2005.



mortality, as kelts in good external condition returned at rates more than an order of magnitude higher than poor-condition fish from all collection sites. Third, known females were both far more abundant than males and were at least two to four times more likely to return as repeat spawners. Fourth, proportionately more wild than hatchery fish and more small than large fish returned, particularly from upstream release groups. Finally, both outmigration year and timing appeared to influence return rates for all groups, with relatively higher returns for earlier outmigrants, and rates differing among years by a factor of two to three at each site.

At the individual fish level, almost all of the kelt characteristics associated with repeat spawning migrations in the Columbia system have parallels in other populations and regions. The tendency for female-dominated iteroparity, for example, is widespread among iteroparous salmonids. This has been attributed to greater reproductive competition among males and subsequently higher postspawn male mortality (e.g., Jonsson et al. 1991; Fleming and Gross 1994). Typical iteroparous females also have fewer individual spawning bouts than males and relatively limited investment in postspawn nest defense, behaviors that result in shorter residency times and higher postspawn survival (Burgner et al. 1992; Fleming 1996; Lohr and Bryant 1999). The tendency for higher returns by smaller steelhead kelts is a trait shared with many Atlantic salmon (*Salmo salar*, Jonsson et al. 1991), brown trout (*Salmo trutta*, Jonsson and L'Abèe-Lund 1993), and Arctic char (*Salvelinus alpinus*, Dutil 1986) populations. Size-related survival may signal some of the same selective pressures that favor large body size in semelparous species (e.g., large body size evolved as migration difficulty increased) as well as a reduced ability for larger fish to restore energy lost during spawning (Crespi and Teo 2002; Kinnison et al. 2003). On average, the largest kelts we examined were from the Snake River, where the life history of many Clearwater and Salmon river steelhead includes an additional ocean year (Busby et al. 1996). Apparent size-related kelt mortality may therefore also be a function of spawner age. Notably, Clearwater and Salmon river steelhead are relatively abundant among Snake River stocks, but were grossly under-represented in kelt genetic samples at Lower Granite Dam (Narum et al. 2008). This suggests initially high postspawn mortality

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(i.e., on or near spawning grounds) for these large-bodied steelhead, consistent with a more semelparous strategy.

In preparation for ocean reentry, kelts must undergo a series of physiological, behavioral, and morphological changes akin to those for juvenile salmonids ("smoltification," reviewed in McCormick et al. 1998). The most visible manifestation of this process is a coloration shift towards the cryptic, silvered appearance typical of pelagic ocean fishes. Smoltification is energetically demanding (Wedemeyer et al. 1980), which may explain why steelhead kelts in poor physical condition were disproportionately darkly pigmented (also see Evans 2003). Not surprisingly, bright kelts in good condition were far more likely to return on repeat migrations. Kelt condition and color metrics may therefore be reasonably good surrogates for kelt energetic reserves and overall physiological readiness for seaward migration and survival. The relative effects of physical condition and physiology on kelt survival and iteroparity could be directly tested by nonlethally measuring kelt energetic reserves (e.g., using a microwave lipid meter), osmoregulatory capability (e.g., Na^+/K^+ ATPase activity), and (or) stress hormones levels (e.g., plasma cortisol) (Congleton et al. 2000; Wagner and Congleton 2004; Cooke et al. 2005).

In smolts, the highest outmigration survival typically occurs when physiological changes coincide with optimum environmental conditions (i.e., temperature, photoperiod, and flow) and upon ocean entry (McCormick et al. 1998). A lack of synchrony during these optimal windows reduces smolt survival and subsequent adult return rates. We expect a similar set of optimal conditions exists for kelts, and outmigration timing and river and ocean conditions may be critical components affecting kelt survival and subsequent repeat spawning events. However, the influence of outmigration river environment on subsequent iteroparity remains unclear. In this study, returns by good condition kelts were much more variable across years (differing by a factor of >2) than were river flow and temperature conditions during outmigration. Wertheimer and Evans (2005) provided evidence for lower kelt outmigration survival in low-flow years, but relationships between river conditions and return rates were inconsistent in our exploratory analyses. This may have been because kelts encounter a wide range of conditions during outmigration — making it difficult to select appropriate metrics — or because river environment or dam operations affect iteroparity at finer scales than we considered. It is also possible that other factors, like ocean conditions, mask the effects of outmigration river conditions. For example, Wertheimer and Evans (2005) reported relatively high downstream survival of 15.6% for Snake River kelts in 2002, yet only ~1% of good-condition fish returned from that sample.

We did find that early-migrating kelts returned at relatively higher rates, a somewhat unexpected result given that bright kelts were increasingly abundant as migrations progressed and peak kelt passage was not observed until midseason (i.e., late April and May). Assuming past selection for migration timing (e.g., Quinn et al. 2000), we expected kelt survival to be highest during peak passage, particularly as this time period is closer to the spring flood. However, the benefits of migrating during historically optimal times may be offset by greatly reduced kelt migration speeds in this impounded system. If there are strong selection pressures for rapid outmigration and ocean reentry timing, the substantial reductions in Columbia River runoff volume and velocity (e.g., Quinn and Adams 1996; Robards and Quinn 2002) could explain the apparent asynchrony between the kelt migration peak and higher iteroparity for early migrants. Alternately, early migrants may have had greater energetic reserves, may have originated from populations more likely to repeat spawn, or may have encountered more favorable river or ocean conditions. A combination of factors likely influenced the reported return rates, and we emphasize that environmental hypotheses remain largely untested.

Our fourth hypothesis, that breeding interval of repeat migrants would differ with kelt outmigration timing and distance, was supported by the data. Compared with kelts that spent a winter at sea (or in the estuary) before returning (i.e., skip repeat spawners), consecutive spawners outmigrated earlier as kelts, were larger, were disproportionately collected at the downstream sites, and returned relatively later in the fall. These patterns likely reflect the time kelts require to restore lost somatic and gonadal energy reserves (Niemelä et al. 2006). Early Columbia River outmigrants may have adequate time to complete this process in a single season, whereas late outmigrants that miss productive ocean conditions in spring may require additional residency. Recovery times may also differ as a function of freshwater migration distance or difficulty (Schaffer and Elson 1975; Willson 1997), perhaps explaining why Snake River kelts overwintered at substantially higher rates than kelts collected at lower Columbia River sites. On average, about 4 months elapsed between hydrosystem detections for consecutive repeat spawners compared with ~15 months for skip repeat spawners. The latter breeding interval is somewhat more typical among anadromous, iteroparous species (Dutil 1986; Jonsson et al. 1991; Behnke 1992).

The general iteroparity and migration timing patterns described above were interrelated in complex ways. For example, kelt coloration and condition varied seasonally and differed between sexes and between wild and hatchery fish (also see Evans et al. 2004*a*). River conditions and dam operations also varied within season, potentially affecting kelt passage routes and survival probabilities (e.g., Wertheimer and Evans 2005). The observational nature of this study made these layers of complexity particularly challenging to untangle, as there were no control groups and potentially important, unmeasured explanatory variables (e.g., ocean effects). The statistical results were therefore useful for identifying broad patterns (i.e., the importance of kelt condition and sex and also migration timing and distance), but these models oversimplify the processes affecting iteroparity and should not be used to infer causality.

We also reiterate that our estimates apply to a broad amalgam of Columbia and Snake river steelhead, potentially representing 30 or more distinct spawning populations upstream from John Day Dam (Brannon et al. 2004; Keefer et al. 2008*a*). Inferences drawn from the aggregate should not be applied to individual stocks given probable among-population variability in spawner age, sex ratios, initial post-spawn survival, and (or) iteroparity. As an example, genetic analyses of kelts collected at Lower Granite Dam showed Snake River populations did not contribute to kelt outmigra-

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tions in proportion to population size or distance to spawning grounds (Narum et al. 2008). This implies that repeat spawning rates for individual stocks may be much higher or lower than aggregate estimates, particularly for kelts passing lower Columbia River dams, where the mix of Snake, mid-Columbia, and upper Columbia river fish is most diverse.

Conservation and management implications

Iteroparity estimates for the aggregate Columbia River samples (5%–6%, across years) were comparable with rates for British Columbia steelhead (Withler 1966), but were generally lower than those reported across a variety of life history types in Washington (7%–11%), Oregon (11%–21%), California (17%–23%), and Alaska (21%–51%) (Shapovalov and Taft 1954; Busby et al. 1996; Lohr and Bryant 1999). Four or more spawning events have been noted in some of these populations, whereas only two steelhead were recorded on a third spawning migration in our study. The iteroparity estimate for Snake River fish (~1%) was among the lowest recorded for any steelhead population and places this group at the low end of the iteroparity continuum for anadromous salmonids (i.e., Fleming 1998). The low rates may be attributable to energetically demanding migrations that favor high single-episode reproductive investment (Crespi and Teo 2002; Fleming and Reynolds 2004) and may have been the norm historically given that interior Columbia River populations have among the longest freshwater migrations recorded for the species (Busby et al. 1996).

An essential management challenge for Columbia River Basin kelts is to reduce the direct and indirect mortality risks associated with dams and reservoirs. In many respects, these risks parallel those for salmonid smolts, with direct mortality hazards including contact with turbine blades, rapid pressure changes in powerhouses, and trauma from passage over spillways (e.g., Čada 2001; Ferguson et al. 2005). A partial list of indirect effects includes energetically costly migration delays, accumulated physiological stress, and possible asynchrony between migration timing and readiness for ocean entry (Venditti et al. 2000; Budy et al. 2002; Schaller and Petrosky 2007). Bypass systems and operational changes designed for smolts (e.g., increased spill during outmigration) may benefit kelts, but these assumptions have rarely been directly tested. The large numbers of kelts collected in juvenile bypass systems and kelt use of a surface-flow bypass structure at Bonneville Dam (Wertheimer 2007) indicate that these facilities can be effective kelt passage routes. Similarly, kelts and prespawn adults pass downstream via spillways when adequate flow is available (Boggs et al. 2004; Wertheimer and Evans 2005). However, understanding route-specific mortality risks, relative use among routes, and effects of route on respawning success will require further investigation.

Broader changes in river environment, such as reduced peak flows during outmigration, are difficult to evaluate without historic data. However, downstream kelt migration rates in the impounded Columbia River system of 13–16 km·day^{-1} in Snake and upper Columbia river reaches and 38 km·day^{-1} in lower Columbia River reaches (Wertheimer and Evans 2005; English et al. 2006) are well below the mean rates these authors report in the Skeena River (42–54 km·day^{-1}), Fraser River (100 km·day^{-1}), and in unimpounded Columbia River reaches (99–111 km·day^{-1}). These data were all collected in low-gradient mainstem reaches, suggesting passage barriers or reduced water velocity in reservoirs delayed outmigration. Directly testing the relationship between outmigration delay and the likelihood of repeat spawning would require large sample sizes but may offer important guidance on flow augmentation or operations-related mitigation.

Two additional strategies to increase iteroparity in Columbia River summer-run steelhead are currently being evaluated. First, a kelt transportation program seeks to circumvent mortality risks associated with dam and reservoir passage by collecting and then transporting kelts downstream in barges or trucks. This effort is modeled after the smolt transportation program, a long-standing Snake River salmonid recovery effort (Ruckelshaus et al. 2002; Buchanan et al. 2006). In a transportation evaluation run concurrently with this project, transport benefits were highest for kelts with the longest migrations (i.e., those collected at Lower Granite Dam) and for wild-origin females (Evans et al. 2008). A second mitigation strategy, kelt reconditioning, involves culturing captive postspawned fish as they reinitiate feeding and redevelop mature gonads. Reconditioning has been successful with Arctic char (Boyer and Van Toever 1993), Atlantic salmon (Johnston et al. 1990; Crim et al. 1992; Moffett et al. 1996), and steelhead (Hatch et al. 2004). Our results suggest targeting females and (or) wild fish in these efforts may provide cost-effective recovery benefits.

In summary, the expression of iteroparity among interior Columbia River steelhead has persisted despite decades of impoundment-related selection pressures against this life history type. This is perhaps unsurprising given the broad phenotypic and reproductive plasticity of *O. mykiss* (e.g., Behnke 1992). The presented data suggest that hundreds to thousands of repeat spawners return to the Columbia River each year, with dispersal throughout the interior basin. Allocations of these fish among the basin's threatened populations and their relative reproductive contributions are currently unknown. However, the results show that repeat spawners are disproportionately female and of wild origin. These are desirable traits given greater reproductive success in wild steelhead (e.g., Chilcote 2003; Araki et al. 2007*a*, 2007*b*) and the tendency for females to be limiting in male-skewed populations (Fleming and Reynolds 2004). Efforts to recover protected populations in the basin should include provisions to promote the demographic, genetic, and stabilizing effects of iteroparity and, more generally, to encourage the full expression of steelhead life history diversity.

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Process-based modelling of decadal trends in growth, survival, and smolting of wild salmon (*Salmo salar*) parr in a Scottish upland stream

William S.C. Gurney, Philip J. Bacon, Graham Tyldesley, and Alan F. Youngson

Abstract: This paper reports a new model of the freshwater stages of an anadromous fish, at the core of which is a stochastic description of the size-at-age dynamics of a growing cohort. Emigration is assumed to require the individual to exceed a threshold size at a critical time of year, thus making the distributions of survival to, and age at, smolting emergent properties of the model. The model is applied to a long-term data set on juvenile Atlantic salmon (*Salmo salar*) in the Girnock Burn, Scotland, to understand the role played by decadal temperature trends in generating changes in smolt production and age distribution. We conclude that changes in age at smolting are compatible with causation by shifts in the temperature regime. However, the large attenuation between a dramatic fall in spawner numbers and a relatively minor diminution in total smolt production does not result from the physiological effects of temperature but is rather a result of strongly density-dependent mortality between the deposition of ova and the appearance of catchable fry the following summer.

Résumé : Notre étude présente un nouveau modèle des stades d'eau douce d'un poisson anadrome qui contient comme élément central une description stochastique de la dynamique de la taille en fonction de l'âge chez une cohorte en croissance. On présume que l'émigration n'est possible que lorsqu'un individu a dépassé une taille seuil à un moment critique de l'année, ce qui fait que les distributions des survies jusqu'à la transformation en saumoneau et des âges lors de cette transformation sont des propriétés émergentes du modèle. Nous utilisons le modèle pour l'analyse qu'une banque de données à long terme sur les jeunes saumons atlantiques (*Salmo salar*) de Girnock Burn, Écosse, afin de comprendre le rôle joué par les tendances décennales de température dans les changements survenus dans la production de saumoneaux et la distribution des âges. Nous concluons que les changements observés dans l'âge de la transformation en saumoneau sont compatibles avec une causalité reliée aux modifications du régime thermique. Cependant, la forte atténuation entre la chute spectaculaire des nombres de reproducteurs et la diminution relativement mineure de la production totale de saumoneaux n'est pas due aux effets physiologiques de la température, mais s'explique plutôt par la mortalité fortement dépendante de la densité entre le dépôt des oeufs et l'apparition d'alevins récoltables l'été suivant.

[Traduit par la Rédaction]

Introduction

Overview

Most salmon species are anadromous, and many form the target of economically important sport and commercial fisheries. Apart from generalized restriction of catches, management actions on salmon populations at sea are complicated by jurisdictional issues and the complexities of differentiating breeding stocks. In contrast, the life-history stages between return of the adults to the river of their birth and emigration of their offspring to sea as smolts are readily accessible.

Effective management of the riverine component of a salmon stock requires an understanding of a complex of processes. The best studied of these is parr growth, which has been the subject of a number of laboratory investigations (Elliott and Hurley 1997; Metcalfe 1998), as well as observation and empirical analysis in the wild (Erkinaro and Niemela 1995; Strothotte et al. 2005; Jutila et al. 2006). Factors known to affect freshwater growth of Atlantic salmon include temperature, food availability, and density (Gibson 1993; Elliott and Hurley 1997; Grant et al. 1998). A relationship between mean age at smoltification and the combination of temperature and day length has been demonstrated across the range of Atlantic salmon by Metcalfe and Thorpe (1990).

Despite this level of empirical study, no process-based

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model is available relating smolt output to the number of adults returning to spawn and the physical and biotic environment experienced by their developing offspring. The stochastic model framework described by Gurney and Veitch (2007) and Gurney et al. (2007) provides a natural platform upon which such a model can be constructed. In this paper, we report a unified dynamic description based on this framework, which covers the entire river phase of the life of an anadromous fish, including recruitment of fry from previously deposited ova, stochastically varying growth and survival of juvenile individuals, and emigration to sea.

We apply this model to a four-decade series of observations of juvenile Atlantic salmon in the Girnock Burn in northeastern Scotland. These data show the number of female spawners decreasing greatly between 1967 and 2003, while smolt production remains almost constant. Over the same period, the mean age at smolting has decreased markedly. The proximate question to which we address ourselves is one that only a process-based model can answer, namely, the relative importance of temperature trends and density-dependent regulation in maintaining smolt production against a large drop in spawner numbers. We are able to conclude that the observed changes in age at smolting are entirely consistent with temperature-induced shifts in growth performance. However, the concomitant changes in survival from fry to smolt, while helpful, play a relatively minor role in maintaining total smolt production compared with the density-dependent regulation evident between ova and fry and the beneficial effects of a recent policy of supportive manipulation of ova distribution within the stream.

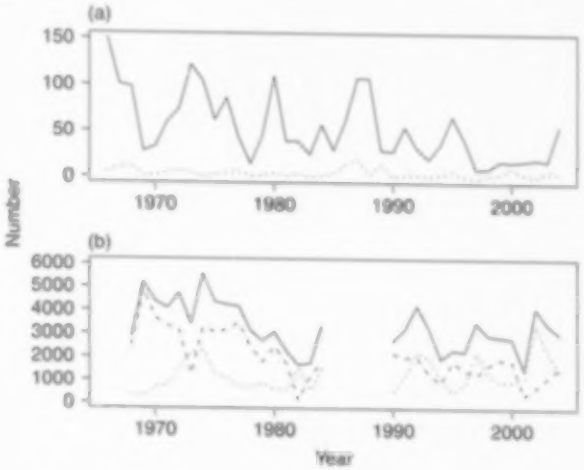
The data

The Girnock Burn is a 9.5 km long, upland tributary of the River Dee, Aberdeenshire, Scotland, the hydraulic characteristics of which have been described by Malcolm et al. (2002). It is an active spawning ground for Atlantic salmon, the spawning habitat use of which has been documented by Moir et al. (1998). In the mid-1960s, a pair of fixed traps was constructed by Fisheries Research Services Freshwater Laboratory (FRS-FL) staff at Littlemill, near its confluence with the main stem of the Dee. The adult trap catches all returning adults, which are counted and sexed, their fork lengths are measured, and their scales are taken for age determination, before release upstream. The smolt trap catches a large proportion of emigrating juveniles, which are counted, their fork lengths are measured, and one in five is scaled for age determination, before release downstream.

For the avoidance of ambiguity, we define river age as the time from 1 January in the year in which the individual hatched. Using this definition, juvenile salmon aged between one and four years emigrate from the Girnock during two main periods (Youngson et al. 1983) — the spring smolting season (March to May) and an autumn parr run (October to December). Tagging studies (Youngson et al. 1994) show the autumn parr to be seaward migrants that are closely associated with the following smolt run and that contribute roughly pro rata to subsequent adult recruitment.

Between 1969 and 1980, the smolt trap was manned almost daily. Most fish were found to move out on high flows on dark nights, so more recent practice concentrates daily visits on the periods of adult return and juvenile emigration, with less frequent visits at other times. In spring, the trap rarely spills and loses few fish. During autumn spates, fallen leaves can block the trap, causing uncontrolled spillage and unquantified fish loss. Because of this difficulty, traditional indices of juvenile production at the Girnock have been based on the spring smolt run alone. However, this study is concerned with the overall demographics of the transition from ova to smolts, and so we need to include the autumn (parr) emigrant data. To avoid confusion, we shall refer to all riverine salmon (juveniles and precocious males) leaving the Girnock as emigrants.

Fig. 1. Spawning and emigration in the Girnock Burn between 1967 and 2003. (*a*) Adult female spawners, with grilse indicated by the dotted line and multi-sea-winter fish indicated by the solid line. (*b*) Total emigrant production (solid line), with younger emigrants (river age at emigration <2.5 years) shown by the dotted line and older emigrants (river age at emigration >2.5 years) shown by the broken line.



In common with a number of other salmon rivers in the United Kingdom and elsewhere, the number of multi-sea-winter fish returning annually to spawn in the Girnock (comprising the great majority of the female breeding stock) has shown a dramatic downward trend over the past 40 years (Fig. 1*a*, solid line), with yearly mean returns falling from 83.6 individuals (1966 to 1976) to 24.7 (1990 to 2000). Over the same period, the output of juvenile emigrants from the system has fallen only slightly (Fig. 1*b*, solid line), with a yearly mean of 4310 individuals before 1977 and 2808 individuals between 1993 and 2003.

Thus, over the four decades of this survey, spawner numbers have declined by 70%, whereas smolt production has decreased by only 35%. During the same period, however, the age at smolting of successive cohorts has changed dramatically (Fig. 1*b*, dotted and broken lines). In the late 1960s and early 1970s, the great majority of the emigrants departed from the Girnock at river ages in excess of 2.5 years, whereas by the 1990s, the numbers of emigrants with river ages above and below 2.5 years were approximately equal.

To obtain a more demographically precise view of these changes, we focus on the properties of cohorts (year classes)

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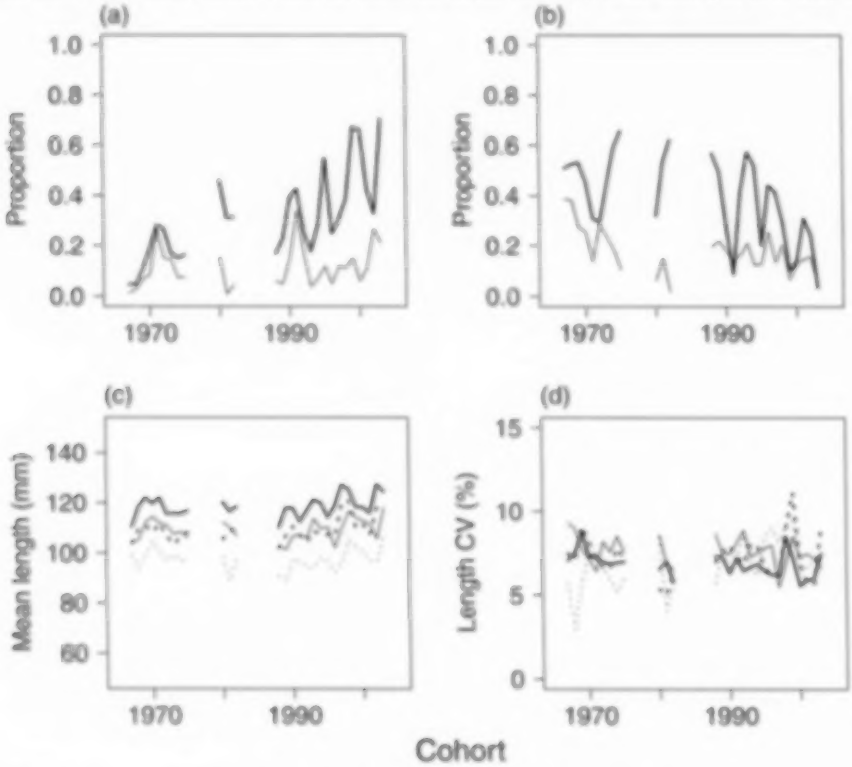
Fig. 2. Trends in emigrant size and age by cohort (year of hatch) between 1967 and 2003. Frames (*a*) and (*b*) show the proportion of the cohort emigrating at different ages. The light line in frame (*a*) shows the proportion emigrating with river ages 1.5 to 2, while the heavy line shows the proportion emigrating aged 2 to 2.5. The light and heavy lines in frame (*b*) show the proportion of emigrants ages 2.5 to 3 and 3 to 3.5, respectively. Frame (*c*) shows the size of individuals from given cohorts emigrating aged 1.5 to 2 (light dotted), 2 to 2.5 (light solid), 2.5 to 3 (heavy dotted), and 3 to 3.5 (heavy solid). Frame (*d*) shows the length variability of the same groups of emigrants.



of individuals. We first show the proportion of total emigrants from each cohort that appear in the trap at a specific age (Figs. 2*a* and 2*b*). We note that to calculate the proportion of the total emigrants from a given cohort departing at a specific age, we must have data for all four years in which members of that cohort might have emigrated. For the 1976–1979 and 1982–1987 cohorts, data for key ages are unavailable or unreliable.

From these plots, we see that over the four decades of the survey, the proportion of the cohort emigrating aged 1.5 to 2 remains about constant, whereas the proportion emigrating aged 2 to 2.5 increases from around 20% to between 40% and 50%, with concomitant decreases in the proportion emigrating later in life. Interestingly, although the mean length of emigrants from a given cohort clearly increases with age, the mean length of emigrants of a given age shows no decadal trend (Fig. 2*c*), a characteristic shared with the length variability (Fig. 2*d*).

For much of the survey period, FRS-FL staff also carried out a late-summer electrofishing campaign in the Girnock during which representative reaches were multiple-pass fished, with all fish caught being counted, all parr and a sample of fry measured for fork length, and all parr scaled for age determination before release. Densities were estimated at each site by depletion calculations. For the period 1998–2000, this programme was expanded to monthly visits to a single station with individuals marked to allow their specific growth and survival to be monitored. Since 2000, a roughly bimonthly programme of electrofishing surveys has been executed covering a more representative sample of habitats within the burn.

Although the continuing programme of electrofishing did not cover the entire burn, the local abundance estimates that it produces can be extrapolated (see Buck and Hay 1984) to infer the total population of different cohorts. This process produces whole-burn population estimates, with year-to-year relativities that are probably reliable but absolute values that are sufficiently uncertain for it to be imprudent to attempt to make age-dependent parr survival a key element in our model, as we would then require a level of self-consistency between the electrofishing population estimates and the trap counts, which experience has shown us to be seldom practical.

Statistical investigations of local growth and survival of fry from recent cohorts (J. Thorley, FRS-FL, Pitlochry PH165LB, Scotland, unpublished data) have suggested that local fry density significantly affects growth and survival in the period leading up to the late-summer survey. We show fry abundances (Fig. 3) derived from the long-term, late-summer electrofishing data by methods described in Buck

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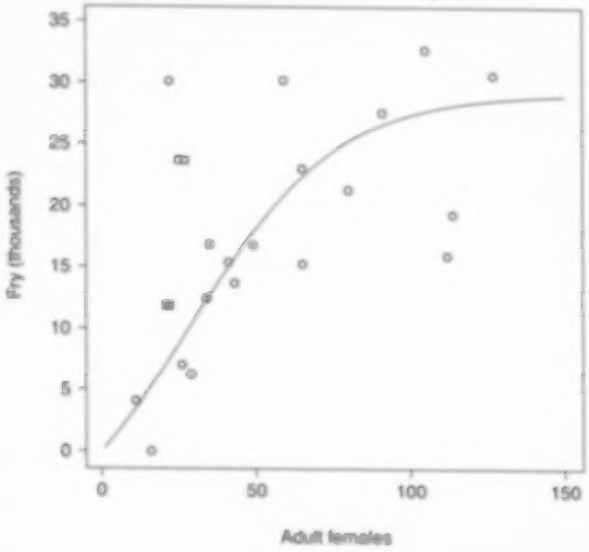
Fig. 3. Historical stock–fry relationship. This figure shows total fry numbers (circles) in the Girnock Burn above the smolt trap, estimated from electrofishing surveys, against total numbers of female spawners (grilse plus multi-sea-winter females). Points from the period 2000–2003, when eggs were stripped from the returning females and "planted out", are shown by squares. The line shows the relation defined in eq. A11, with $F_0 = 8862$, $F_{max} = 29\,212$, and $A_0 = 24$, which yields $R^2 = 0.61$ for the nonplanted-out points compared with 0.45 for a two-parameter linear regression.



and Hay (1984) against spawner numbers. This plot illustrates a stock–recruitment relationship between late-summer fry densities and spawner numbers, the saturating form of which must arise from the complex of density-dependent effects that may occur between adult return to the river and observation of fry in the following year's survey.

Temperature loggers have been deployed for varying durations at various locations in the Girnock during the period of interest. However, only the one at Littlemill has been deployed continuously for the entire period. We show time series (Fig. 4) of the monthly averaged temperatures for four months typical of winter, spring, summer, and autumn (January, April, July, and October), together with regressions against year. We see that although autumn and winter temperatures have decreased by 0.76 °C and 0.57 °C, respectively, between 1970 and 2000, spring and summer temperatures have increased by 1.46 °C and 1.04 °C, respectively, over the same period.

Materials and methods

The model

The model used in this paper is an input–output description of the connection between individuals returning to a subcatchment to spawn and the subsequent emigration of their offspring. The stochastic description of growth around which this model is built is an extension of that used by Gurney et al. (2007) to model the growth of trout and salmon parr. To this growth description (extended to encompass growth of fry after emergence from the gravel) are added descriptions of the deposition of ova and their survival to become fry and emigration of parr to become smolts.

We give an extended mathematical description of this model in Appendix A. In this section, we set out the key properties of the underlying description of growth, discuss the extensions that transform it into a riverine input–output model, and define the parameters that will be used in subsequent discussion.

The model developed by Gurney et al. (2007) describes the dynamics of the length distribution of a cohort of functionally identical individuals growing in a stochastic environment in such a way that mean length at age increases towards an asymptotic value L_∞ according to a von Bertalanffy (1938) pattern. The model's computational efficiency results from a formal description in terms of a development index (q) related to individual length (L) in such a way that a series of equal width classes represents the growth increment achieved by their occupants in a fixed time period (eq. A4).

The cohort description is updated at intervals proportional to the development index class width, dq (eq. A13). Growth stochasticity is incorporated by assuming that in any update interval, an individual either grows enough to put it into the adjacent class (with probability p) or stays the same length (probability $1 - p$). Gurney et al. (2007) showed that mean length at age then follows a von Bertalanffy pattern with realised growth rate γ given by eq. A6.

To model growth seasonality, Gurney et al. (2007) assumed that p had a high value (which we denote by P) between days (of the year) D_g and D_w, whereas for the rest of the year, it is lower by a factor ω. We carry over these assumptions for parr but recognise that fry occupy different habitat from parr and have a longer growing season (J. Thorley, FRS-FL, Pitlochry, PH16 5LB, Scotland, unpublished data). We assume that the summer growth rate for fry differs from that of parr by a factor φ and that their growing season ends on day (of the year) D_w^f.

Gurney et al. (2007) assumed both growth probabilities and realised growth rate to be constant within a season. However, we seek to model temperature effects, so we need to write γ as a function of temperature. Although salmon parr growth rates are known to fall at temperatures in excess of 18 °C (Elliott and Hurley 1997), Girnock temperatures seldom exceed this value (see Fig. 4). We represent γ for both fry and parr by an activated process (eq. A8), with an activation temperature (T_a) driven by temperatures linearly interpolated from the monthly average temperatures at Littlemill. For parr, we assume that the realised growth rate changes by the same factor as the growth probability at the summer–winter boundaries, so we write the scale for the activated process as γ_0 in summer and $\omega\gamma_0$ in winter. For fry, unpublished work on the raw data that underlies that used in this study (J. Thorley, FRS-FL, Pitlochry, PH16 5LB, Scotland, unpublished data) has shown significant density dependence of realised growth rate. Hence, for fry, we assume that very small cohorts have realised growth rate scale $\eta\gamma_0$, but that the scale drops linearly (with slope ξ) as numbers rise.

Two further extensions to the Gurney et al. (2007) struc-

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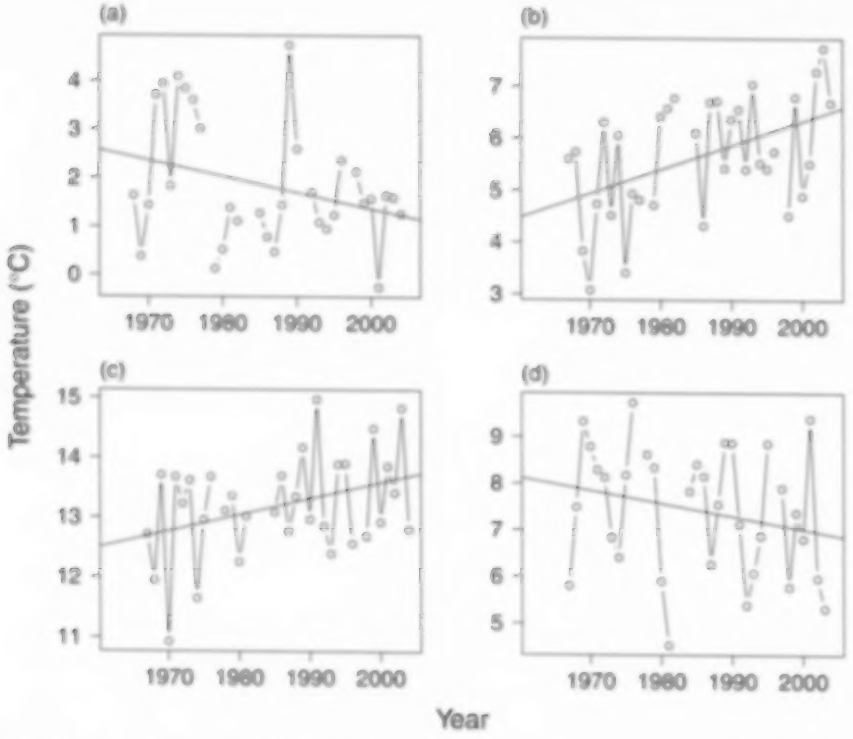
Fig. 4. Monthly average temperatures at Littlemill on the Girnock Burn from 1966 to 2005. Points joined by lines show monthly averaged temperatures (°C). The solid straight line shows a linear regression of month temperature against year: (*a*) January, (*b*) April, (*c*) July, and (*d*) October.



ture are needed to construct the input–output model that we require for this study, namely, recruitment and emigration. To model emigration, we assume that the spring and autumn runs occur instantaneously on days (of the year) D_i^{spr} and D_i^{aut}, respectively. On these days, we assume that there is a length-dependent probability of emigrating that is very low for lengths, well below respective thresholds L_h^{spr} and L_h^{aut}, rising to e^{spr} or e^{aut}, respectively, for lengths well above threshold, with the transition occurring over a length range L_h^{spr} or L_h^{aut}, respectively.

Very little field data are available that we could use to elucidate the part of the life cycle between ova deposition and appearance of the fry in the autumn fry survey. The stock–recruitment relation shown in Fig. 3 demonstrates strong density dependence of survival from ova to fry but gives no hint of the processes that underlie this effect. Hence we arrange for appropriate numbers of fry to arrive at the autumn survey (on day of the year D_{sfs}) by depositing the numbers implied by the stock–recruitment relation (eq. A11) in the system at the time when the alevins are emerging from the gravel (which we denote by D_{hatch}) and making them immortal until D_{sfs}. The newly emerged individuals are assumed to have mean length L_{hatch} and length CV C_{hatch}. This slightly convoluted strategy allows us to model density-dependent fry growth (at least approximately) while avoiding unverifiable detail in the description of fry mortality.

We give a complete list of model parameters, together with their interpretation and best-fit values (Table 1). We can obtain values for some of these parameters from independent data, the literature, or inspection of biological or operational reality. In the first class are the values shown in the caption for Fig. 3, obtained from the data shown in the figure using nonlinear minimization (the Nelder–Mead algorithm implemented in the R routine "optim") of the mean-square deviation of observed and predicted points. In the second are the asymptotic length L_∞ (Gurney et al. 2007), the activation temperature T_a (Elliott 1976), the parr mortality rate (Egglishaw and Shackley 1985), and the growing season limits (Gurney et al. 2007). In the third are the emigrant run timings, which were selected to represent the end of the spring and autumn periods during which emigrants leave the burn and the timing of the late-summer electrofishing campaign.

Data fitting

In the remainder of this paper, we use fits of the model to various subsets of the data both to evaluate parameters we cannot determine independently and to test the model's validity by examining the quality of the best achievable fit. In related work (Gurney et al. 2007), we used various brands of nonlinear minimization for this purpose and determined confidence limits by data resampling. Here we use the Markov chain Monte-Carlo approach to Bayesian inference to determine the full probability distribution for the vector of unknown parameters. For the data discussed here, this

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Table 1. Model parameters, units, best-fit values, and sources.

Parameter	Value	Unit	Interpretation	Source
Population representation				
dq	0.01		Development index class width	Arbitrary
L_∞	200	mm	Asymptotic length in burn	Gurney et al. (2007)
Fry recruitment				
F_0	8862	no. of fry	Stock–recruitment — offset	Fig. 3 fit
F_{max}	29212	no. of fry	Stock–recruitment — maximum	Fig. 3 fit
A_h	24	no. of females	Stock–recruitment — half saturation	Fig. 3 fit
D_{hatch}	90	day (of year)	Hatch day	Youngson et al. (2004)
L_{hatch}	18	mm	Mean length at hatch	Youngson et al. (2004)
C_{hatch}	3	%	Length CV at hatch	Youngson et al. (2004)
Parr growth and mortality				
D_0	72	day (of year)	Growing season — start	Gurney et al. (2007)
γ_0	0.091	%·day^{-1}	Realized growth rate scale	Fitted — Table 3
T_a	11.9	C	Activation temperature	Elliott (1976) and Table 3
P	0.069		Summer growth probability	Fitted — Table 3
ω	0.237		Winter–summer growth probability	Fitted — Table 3
D_∞	188	day (of year)	Growing season — end	Gurney et al. (2007)
m	0.19	%·day^{-1}	Per capita mortality rate	Egglishaw and Shackley (1985)
Fry growth				
η	0.569		Fry–parr realised growth rate scale	Fitted — Table 3
φ	3.07		Fry–parr growth probability	Fitted — Table 3
ξ	3.98×10^{-7}	day^{-1}·fry^{-1}	Fry growth density dependence	Fitted — Table 3
D_{ef}	200	day (of year)	Electrofishing survey	Nominal
D_∞^f	200	day (of year)	Fry growing season — end	J. Thorley, unpublished
Spring emigration				
D_r^{sp}	120	day (of year)	Run	Nominal
L_b^{sp}	102	mm	Sigmoid probability function — centre	Fitted — Table 3
L_w	0.104	mm	Sigmoid probability function — width	Fitted — Table 3
ε^{sp}	1.0		Pr[qualified individual emigrates]	Assumed
Autumn emigration				
D_r^{au}	300	day (of year)	Run	Nominal
L_b^{au}	95.6	mm	Sigmoid probability function — centre	Fitted — Table 3
ε^{au}	0.29		Pr[qualified individual emigrates]	Fitted — Table 3

approach has produced fits comparable with those obtained by nonlinear minimization, together with full information on parameter uncertainties and correlations, at a computational cost very similar to that for a single nonlinear minimization.

Because the computational efficiency of the model evaluation is the key to producing Markov chains of useful length, we use a self-programmed implementation of the Metropolis–Hastings algorithm (Hastings 1970), written in a compiled language (Pascal), in preference to a prewritten application in an interpreted language such as R. Our approach is based on a likelihood function closely related to the error function used in our earlier work. For each point, we calculate a relative absolute error defined as the absolute difference between observation and prediction divided by the 95% confidence range for the observed value. For each curve, we calculate the mean relative absolute error per point. Finally, we calculate the mean of the four curve errors and then determine the likelihood of the observations assuming that the relative absolute error follows a standard Laplace distribution. This formulation, which is equivalent to assuming that the N data points are individually Laplace distributed and using the Nth root of the probability as our likelihood function, enables us to avoid generating computationally unfeasible likelihoods, even from very noisy data.

In almost every case, we know virtually nothing about either the expectation value or the distribution of the unknown parameters, so we employ a prior distribution that expresses very generalized initial knowledge — all parameters must be nonnegative and probabilities must lie in [0, 1]. The proposal distribution echoes the Laplace form of the assumed error distribution, but with a standard deviation for each component chosen to be a standard fraction of a scale vector, normally set equal to the initial parameter values.

During an initial burn-in period (10 000 evaluations for single cohort fits, 1000 for time series fits involving 36 cohorts), the scales are varied so that 50% to 70% of proposed points are accepted, thus resulting in efficient exploration of the posterior distribution. After completion of the burn-in period and finalization of the proposal distribution, a series of evaluations (100 000 for single cohort fits, 20 000 for time series fits) is used to construct the definitive Markov chain. During this process, we record the likelihood associated with each proposed point and use the parameter vector

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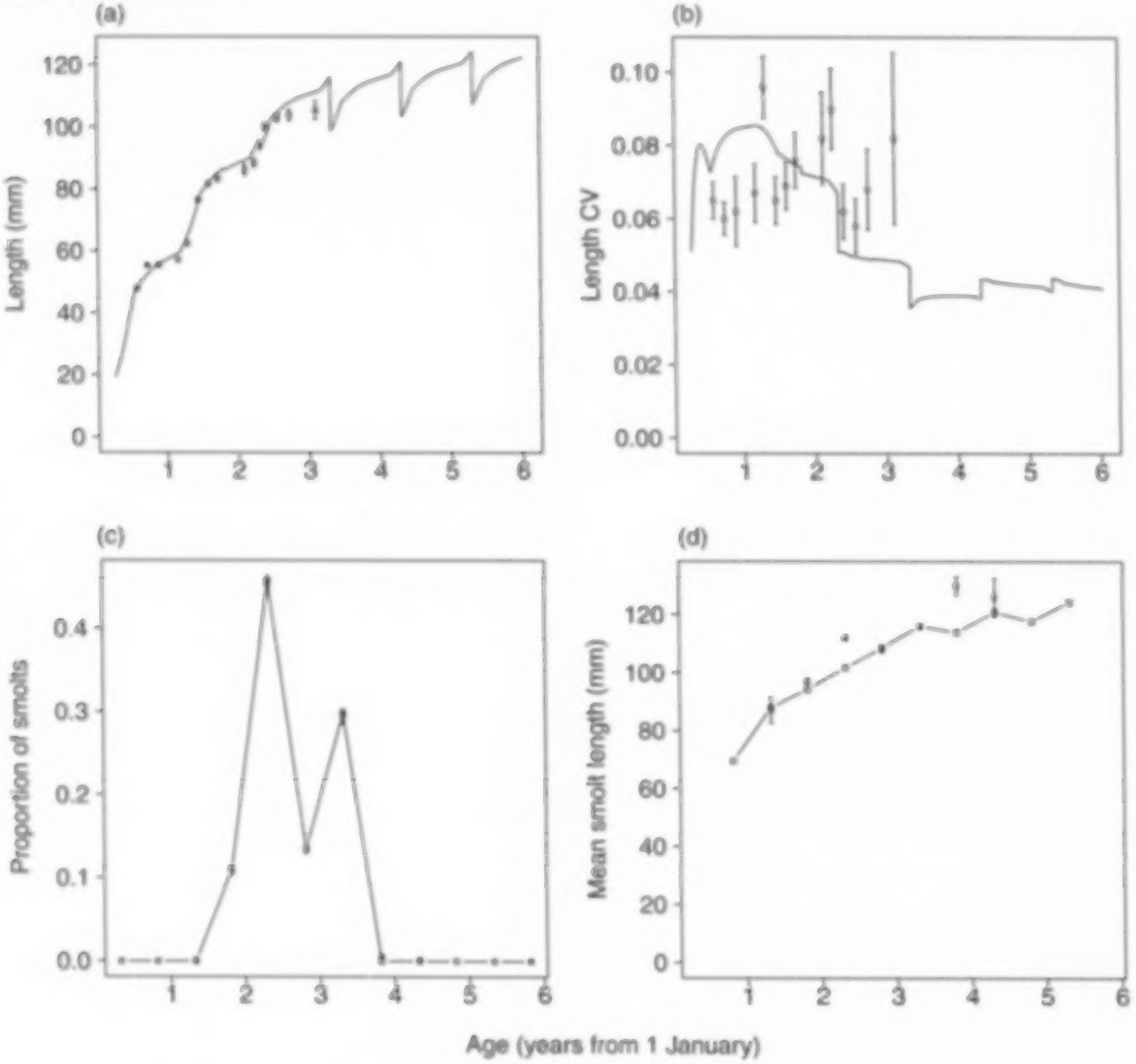
Fig. 5. Best fit to 2001 cohort date. Frame (*a*) shows predicted (solid line) and observed (points) mean length at age. Error bars show 95% confidence range calculated by bootstrap resampling. Frame (*b*) shows predicted (solid line) and observed (points) CV of length at age. Error bars show 95% confidence range calculated by bootstrap resampling. Frame (*c*) shows predicted (points joined by lines) and observed (points with error bars) proportion of emigrants per run. Error bars are calculated assuming that emigration is a binomial process with small success probability, so the variance of numbers observed is equal to the mean. Frame (*d*) shows predicted (points joined by lines) and observed (points with error bars) mean length of emigrants in each run. Error bars are calculated assuming that the observations are normally distributed around the mean.



with maximum likelihood as our best fit. Although we did not use any formal test for convergence of the Markov chain, multiple applications of this process in test cases demonstrated that the results are robust.

Results

Fitting to the 1997–2003 high-resolution growth data

Our first fitting exercise uses high temporal resolution data for cohorts hatched in the years 1997 to 2003 to test model validity and estimate all but one of the parameters in Table 1 for which we do not have independent values. During the latter half of the period for which such data are available, ova planting may have perturbed the fry stock–recruitment relation and (or) the parr mortality rate. The mean and CV of size at age and the mean emigrant length are weakly dependent on mortality and recruitment. Although the numbers emigrating at age are strongly af-

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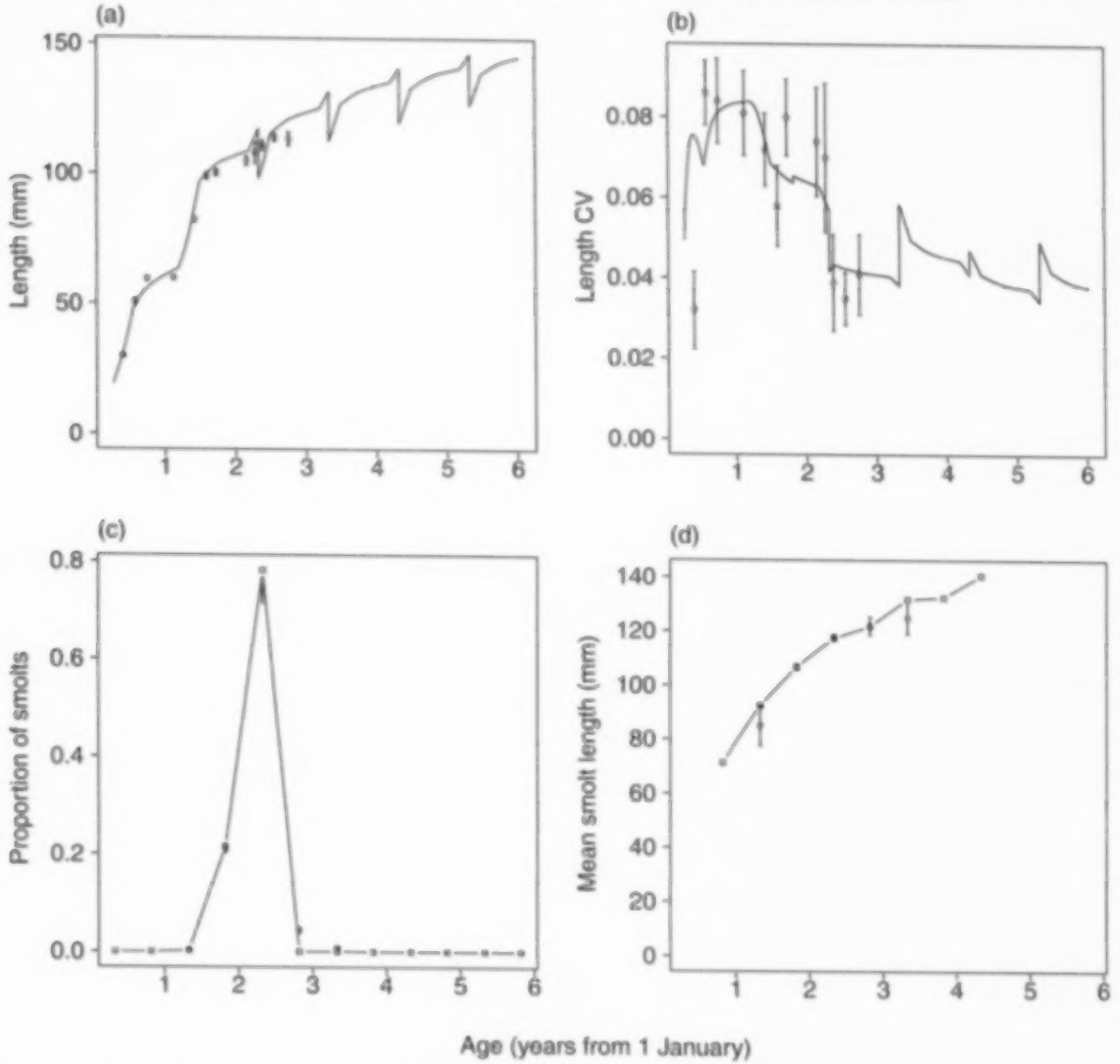
Fig. 6. Best fit to 2003 cohort data. Frame (*a*) shows predicted (solid line) and observed (points) mean length at age. Error bars show 95% confidence range calculated by bootstrap resampling. Frame (*b*) shows predicted (solid line) and observed (points) CV of length at age. Error bars show 95% confidence range calculated by bootstrap resampling. Frame (*c*) shows predicted (points joined by lines) and observed (points with error bars) proportion of emigrants per run. Error bars are calculated as in Fig. 5. Frame (*d*) shows predicted (points joined by lines) and observed (points with error bars) average length of emigrants in each run. Error bars are calculated as in Fig. 5.



fected by both these factors, the proportions departing at given ages are not. Hence, we minimize the effect of using data from cohorts observed during a period of river manipulation by fitting the mean and CV of size at age, the proportion of emigrants in each run, and the mean length of these groups of emigrants — all of which are almost independent of both the stock–recruitment relation and the parr mortality rate.

To investigate the possibility that unmodelled year-to-year changes in the physical and biotic environment are a significant source of year-to-year variability, we fit the model separately to each of the observed cohorts. In such fits, the fry growth rate density-dependence parameter (ξ) is almost completely confounded with the fry-to-parr growth rate scale ratio (η), so we set $\xi = 0$.

For all fitted cohorts, the quality of fit closely resembles those illustrated in Figs. 5 and 6. The mean length-at-age data are well fitted, as are the proportions emigrating at age, despite considerable year-to-year variation in the temporal distribution of emigration (compare Fig. 5*c* and

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Table 2. Individual cohort fits from 1997 to 2003, calculated with the activation temperature T_a = 12 °C) and no density dependence in fry growth (ξ = 0).

Parameter	1997	1998	1999	2000	2001	2002	2003
γ_0	0.070	0.102	0.067	0.096	0.064	0.111	0.122
	(0.07, 0.17)	(0.05, 0.14)	(0.04, 0.08)	(0.07, 0.12)	(0.05, 0.08)	(0.09, 0.12)	(0.07, 0.12)
P	0.016	0.020	0.153	0.089	0.149	0.579	0.049
	(0.01, 0.07)	(0.01, 0.06)	(0.02, 0.45)	(0.01, 0.14)	(0.06, 0.56)	(0.28, 0.83)	(0.02, 0.11)
ω	0.591	0.230	0.445	0.142	0.315	0.108	0.216
	(0.02, 0.60)	(0.04, 0.93)	(0.28, 0.96)	(0.02, 0.30)	(0.19, 0.59)	(0.07, 0.25)	(0.20, 0.53)
η	0.147	0.611	1.158	0.793	1.065	0.551	0.539
	(0.08, 0.37)	(0.36, 1.21)	(0.94, 2.34)	(0.56, 1.20)	(0.93, 1.54)	(0.44, 0.71)	(0.49, 0.92)
φ	0.445	23.94	5.24	6.36	4.83	1.38	15.5
	(0.15, 4.89)	(1.95, 24.3)	(0.70, 26.4)	(4.26, 12.3)	(0.28, 12.4)	(0.32, 1.55)	(3.91, 27.7)
L_0^{spr}	106	110	102	98	98	104	96
	(103, 107)	(108, 115)	(100, 108)	(97, 101)	(97, 101)	(102, 106)	(96, 106)
L_w	0.026	0.65	0.20	0.04	1.36	0.62	1.67
	(0.03, 0.88)	(0.19, 3.13)	(0.03, 0.67)	(0.02, 0.06)	(1.59, 4.10)	(0.13, 1.31)	(0.82, 3.42)
L_0^{aut}	91.5	101	98.9	98.2	91.4	93.4	97.2
	(87, 94)	(98, 109)	(93, 103)	(97, 101)	(91, 99)	(90, 95)	(97, 109)
ε^{aut}	0.20	0.34	0.29	0.40	0.24	0.31	0.19
	(0.15, 0.32)	(0.21, 0.46)	(0.12, 0.33)	(0.29, 0.47)	(0.22, 0.35)	(0.21, 0.38)	(0.18, 0.64)

Note: Best-fit values are given on the first line, with 5 and 95 percentile values in parentheses below. Units as in Table 1.

Table 3. Best-fit values for all fitted parameters.

				Time series, 1967–2003		
Parameter	Interpretation	Unit	Cohort weighted average	5 percentile	Best fit	95 percentile
T_a	Activation temperature	°C	[12.0]	7.7	11.9	14.6
γ_0	Realised growth rate scale	%·day^{-1}	0.090	0.061	0.091	0.102
P	Parr summer growth rate probability		0.058	0.026	0.069	0.155
ω	Winter–summer growth rate probability		0.237		[0.237]	
η	Fry–parr realised growth rate scale		0.569		[0.569]	
φ	Fry–parr growth probability		3.07		3.07	
L_0^{spr}	Spring smolt probability centre	mm	102		[102]	
L_w	Smolt probability width	mm	0.104		[0.104]	
L_0^{aut}	Autumn smolt probability centre	mm	95.6	90.5	92.5	96.9
ε^{aut}	Pr[qualified emigrates] — autumn		0.29		[0.29]	
ξ	Fry growth rate scale density dependence	%·day^{-1}·fry^{-1}	[0.0]	2.8×10^{-8}	3.98×10^{-7}	4.1×10^{-7}

Note: Column four shows weighted averages of the cohort-by-cohort fitted values from the 1997–2003 data. Column six shows best-fit values for a subset of parameters refitted ot the time series input–output data from 1967–2003. The 5 and 95 percentiles for these refitted parameters are given in columns five and seven, respectively. Quantities in columns 4 and 6 shown in brackets were taken as constant for the fitting exercise concerned.

Fig. 6c). The mean smolt length data are also well fitted, with the exception of points generated by very small numbers of fish at the start and end of the sequence of emigration opportunities. The model's performance in mimicking the observed CV of length at age is more mixed. In the case of the 2003 cohort, the match between predictions and data is broadly good. However, for the 2001 cohort, there is an initial sequence of rising CV with age, which the model struggles to reproduce.

The best-fit values for each cohort and the 5 and 95 percentiles are shown (Table 2). Examination of the Markov chains from the estimation process shows that most parameter estimates are only weakly correlated with others in the parameter vector, the only exception being the realised growth rate scale γ_0, which is negatively correlated with the winter-to-summer growth rate scale ratio, ω (see Appendix B). This correlation occurs because low winter and high summer growth rates produce the same yearly growth increment as higher winter and lower summer growth rates. It is particularly evident where the data do not define the summer–winter growth difference precisely.

We show a canonical parameter set (Table 3) constructed as a weighted average over cohorts with weights inversely proportional to the range between the 5 and 95 percentiles. Comparing this canonical parameter set (Table 3, column 4) with the best-fit parameters (Table 2) reveals some robust patterns.

Parr realised growth rate scales (γ_0) for individual cohorts are consistent with the canonical average value of 0.09%·day^{-1}, except for 1999 and 2001, which show significantly lowered values. Individual-to-individual variability in parr growth is generally high, as indicated by a canonical

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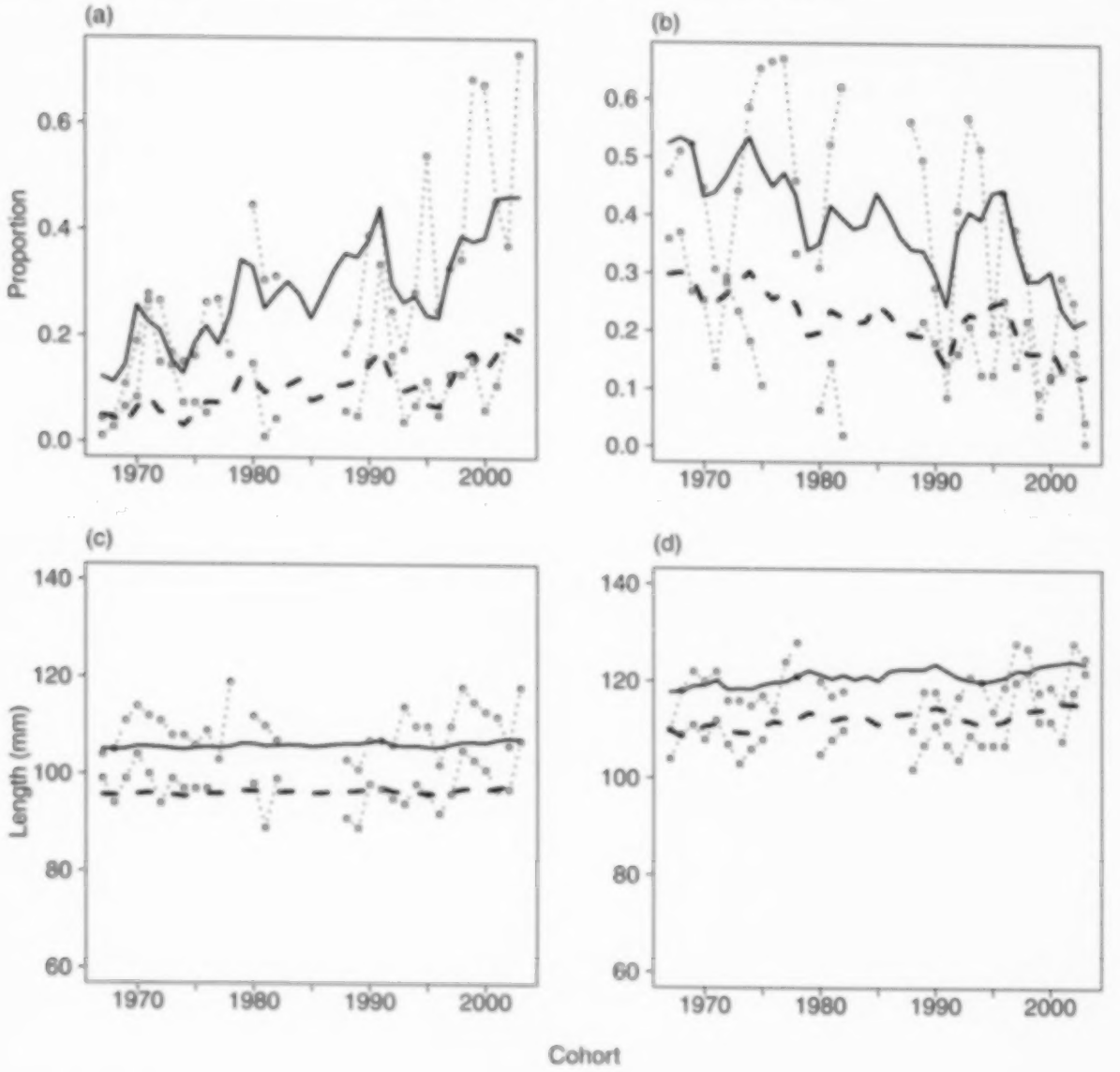
Fig. 7. Best fit to long-run time series. (*a*) Predictions (bold broken line) and observation (circles) of the proportion of all emigrants from cohorts from 1967 to 2003 leaving in the autumn of the year in which they have river age 1, together with predictions (bold solid line) and observations (squares) of the proportion leaving the following spring. (*b*) Predictions and observations, similarly distinguished, for the proportions emigrating in the autumn of the year in which they have river age 2 and the following spring. (*c*) Predictions and observations for the mean length of the subcohorts portrayed in *a*. (*d*) The mean length for the subcohorts portrayed in *b*.



value of $P = 0.058$, the exception being 2002, with a 5 percentile P value of 0.28.

The step-like character of the mean length at age curves and the depression of winter growth below the level that temperature alone would imply are reflected in the ratio of winter to summer growth rate scales. These are broadly consistent with a canonical value of $\omega = 0.237$, the only exception being 1999, with a 5 percentile value of 0.28.

Estimates of the ratio of the realised growth rate scales of fry and parr (η) vary significantly between cohorts. The weighted average value over all cohorts is 0.569, but only three of the seven individual estimates are consistent with this value, with two of the remainder being higher and two lower. However, only two cohorts have $\eta > 1$, and even then the confidence limits encompass unity. We conclude that fry growth is generally slightly slower than that for parr.

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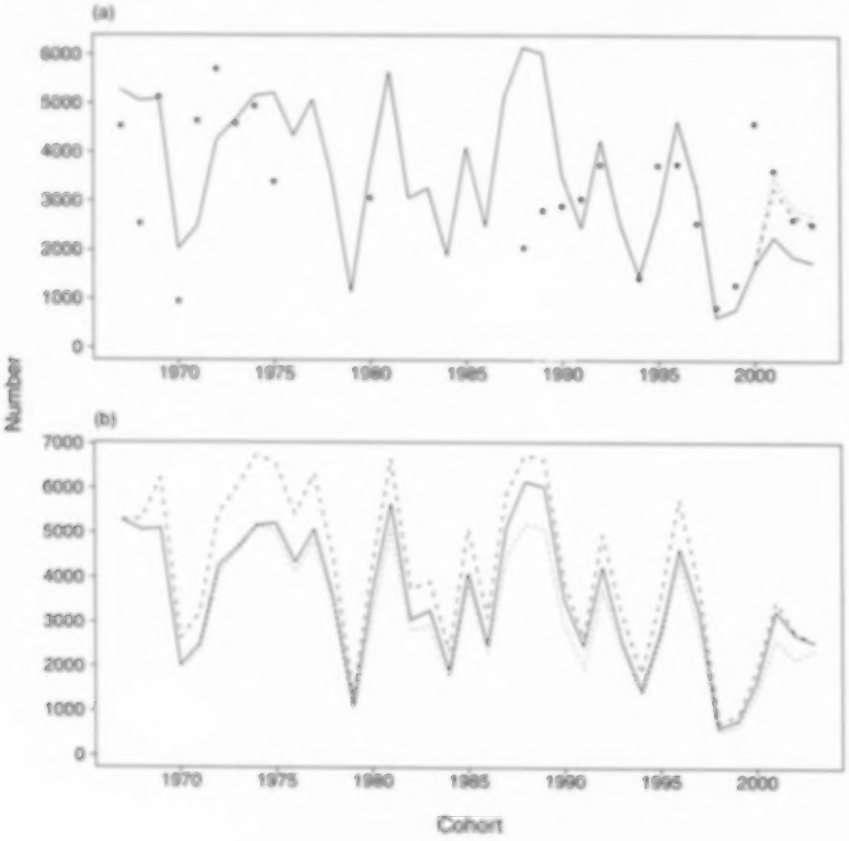
Fig. 8. Postdicting total emigrant production. Lines show predicted total emigrants per cohort. Solid circles show observed total emigrants per cohort. (*a*) The solid line shows predicted emigrants per cohort with parameters from Table 1. The broken line shows predictions with cohorts from 2001 having a stock–recruitment half-saturation spawner population $A_0 = 17$ for cohorts from 2001 onwards. The dotted line shows predictions with all cohorts from 2001 on having parr mortality rate $m = 0.13\%\text{-day}^{-1}$. (*b*) The solid line shows predictions with parameters in Table 1 except that cohorts from 2001 on have $A_0 = 17$, driven by monthly average temperatures illustrated in Fig. 4. The broken and dotted lines show an identical run except that all cohorts are subjected to temperatures from the equivalent period in 1969 (dotted line) or 2003 (broken line).



Individual-to-individual variability in fry growth is clearly less than that for parr, with all the individual cohort estimates for the growth probability ratio, φ, being consistent with the weighted average value of 3.07.

The estimated values for the spring emigration threshold, while individually well defined, show significant variation from year to year. Again three of the seven cohorts are consistent with a canonical value of 102 mm, while two are clearly higher and two lower. All but one of the estimated values for autumn emigration thresholds are lower than the spring estimate for the same cohort, and again three cohorts are consistent with the weighted average value of 95.6 mm, while two of the remainder are lower and two higher.

The estimates for the transition width (L_ω), while varying considerably, are all small, with no best-fit value exceeding 2 mm. The cohort estimates for the proportion of qualified autumn emigrants that actually leave are all consistent with a weighted average value of 0.29.

Fitting to the 1967–2003 time series

The input–output data for the period 1967–2003 are sufficiently rich for the goodness of fit obtainable by optimizing a small group of unknown parameters against it to provide a valid test of our model. We first focus on trends in size and age at smolting, i.e., on the proportion of the total emigrants from a given cohort leaving in a given run, and the mean length of such groups of emigrants (Fig. 2).

Our fitting strategy will be to estimate the only parameter for which we so far have neither a literature-derived nor a fitted value (the fry density-dependence slope, ξ) together with the activation temperature (T_μ), the baseline growth rate scale (γ_0), the baseline growth probability (P), and the difference between the spring and autumn emigration thresholds.

Using the same MCMC approach as before, we produce a best fit (Fig. 7) that captures both the trend and the main features of the year-to-year variations in proportional emigration. There are some year-to-year variations that the model

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fails to capture, for example, periods of above-trend emigration in the spring of the year in which the cohort's river age is three years and concomitant below-trend emigration of the previous autumn. Experiments with model variants without fry growth density dependence (not shown) displayed observably larger deviations from historical behaviour.

Although the model fails to postdict much of the year-to-year variability in size at smolting, it correctly captures both the trend and the age relativities of this quantity — a property noticeably absent from fitting experiments (not shown) with versions of the model with the spring and autumn smolt characteristic length (L_0^{spr} and L_0^{aut}) set equal.

The best-fit parameters for the fit illustrated (Fig. 7) are given (Table 3, column six). From this we see that the best-fit value of T_μ is very close to the Elliott (1976) value of 12 °C. Neither the parr summer growth rate scale (γ_0) nor the growth probability (P) differ significantly from the canonical (weighted average) values for the individual cohort fits (Table 3, column 4). Although the confidence range for the re-estimate of the autumn smolting threshold (L_0^{aut}) encompasses the canonical value, we note that predictions using the re-estimate are visibly more faithful to the data than those using the canonical value. Finally, the fry growth density-dependence parameter ξ, while numerically small, is significantly different from zero. Its best-fit value implies a reduction of about 40% in fry growth rate if the burn is fully populated with fry.

Postdicting emigrant production

A simple re-presentation of the results used to generate Fig. 7 gives us a prediction of the decadal variation of total emigrant production that we can compare with the data (Fig. 8*a*, solid line). In contrast to the calculation of proportional emigration, this postdiction depends critically on the model inputs in the form of adult spawners, the stock–fry recruitment curve, and the parr mortality, so the generally good correspondence between data and predictions indicates the validity of both the model and the parameter values in Table 1.

However, we note that for cohorts from 2001 onwards, when ova planting was undertaken, the smolt output is considerably underpredicted. This seems likely to result from changes in fry or parr mortality, consequent upon differences between a uniform planted ova distribution and a more clumped natural distribution, or from reduced egg mortality during incubation relative to natural conditions. To illustrate the likely magnitude of the changes involved, we also show (Fig. 8*a*) the trajectories that would result from cohorts from 2001 onwards having reduced parr mortality (m = 0.13%·day^{-1}, dotted line) or a stock–recruitment curve that rises more rapidly at low spawner levels (A_b = 17, broken line).

We illustrate a simulation experiment (Fig. 8*b*) designed to distinguish between year-to-year variability produced by variations in spawner numbers and variations driven by year-to-year temperature changes. We repeat the best-fitting run (Fig. 8*a*, parameters from Table 1 except that cohorts from 2001 have A_b = 17) but with the temperature cycle in all years set equal to that in 1969 (Fig. 8*b*, dotted line) or 2003 (Fig. 8*b*, broken line). We see that both these changes produce changes of around 20% in smolt production in some years, but neither has any pronounced effect on the predicted trend. We conclude that the decadal temperature trends illustrated (Fig. 4), while clearly implicated in the changes in age at smolting (Fig. 7), have had relatively minor effects on total smolt production.

Discussion

In this paper, we describe a stochastic model of salmon parr recruitment, growth, survival, and smolting that we have applied to four decades of data from a Scottish upland stream (the Girnock Burn). From this we draw two principal conclusions: first, that trends in age at smolting are compatible with causation by a temperature-dependent growth rate in the form of an activated process with an activation temperature close to that given for *Salmo trutta* by Elliott (1976); and second, that the remarkably small change in total smolt output, in the context of a 70% decrease in spawner input, must be ascribed to strong density dependence in ova or fry survival — caricatured in this model by a spawner–fry stock–recruitment relation. This relation appears to have been altered by the ova-planting programme affecting cohorts hatched from 2001 onwards.

We note that the canonical parameter set given in Table 1 contains values that, where they can be compared with biological reality, are broadly reasonable. Interestingly, although the summer von Bertalanffy growth rate scales for fry and parr are similar, the growth probabilities for the two stages are very different. This argues that fry growth proceeds at much the same relative rate as that of parr but is much less variable. In this context, we note observations made at FRS-FL (J. Gilbey, FRS-FL, Pitlochry, PH165LB, Scotland, unpublished data) that suggest that a compensation mechanism operates on alevin growth.

Our model was driven by monthly averaged temperatures at a single station (Littlemill). Recent observations (Malcolm et al. 2002) have shown differences in daily mean temperature of several degrees between this station and the upper reaches of the catchment. However, the upstream temperatures were shown to be highly correlated with those at Littlemill, so we believe that monthly averaged temperatures at Littlemill form a suitable index for temperature trends over the parts of the main stem where maximum spawning activity is located. We also note that in our model, growth rate variability consequent on short-term local variability and spatial heterogeneity are both subsumed within the stochasticity of the growth process.

The activated process form that we have used for the temperature dependence of the growth rate scale, although highly appropriate for the basal metabolic rate that it nominally describes (see Gurney et al. 2007), is incompatible with data, given by both Elliott and Hurley (1997) and Forseth et al. (2001), showing that growth rates plateau at around 18 °C and begin to drop when temperatures much exceed this value. In waters where such temperatures routinely occur, this aspect of the model would certainly require modification. However, in the Girnock, where weekly average temperatures seldom exceed 18 °C, we do not believe that the robust deviations between observation and postdictions based entirely on temperature-dependent growth are caused by such infelicities.

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Managers of Atlantic salmon (*Salmo salar*) fisheries face two major concerns. Increased marine mortality (ICES 2006) is jeopardising many populations by reducing the number of adults returning to breed. More recently it has been accepted that climate change is likely to alter the production of salmon smolts from freshwater (McCarthy et al. 2001). The domed nature of the temperature dependence of individual growth performance observed in tank experiments (Elliott and Hurley 1997; Forseth et al. 2001) has led to qualitative suggestions that waters with current average temperatures below 16 °C will show increased production as a result of increasing temperatures (e.g., Langan et al. 2001), whereas waters with higher current temperatures will show production decreases (e.g., Swansburg et al. 2002). However, we note that these hypotheses depend on an unchanging food environment (or at least a continuing absence of food limitation).

Quantitative assessment of the changes in freshwater production that may flow from water temperature changes requires a detailed understanding of the complex of processes governing the development and survival of salmon parr from ova to smolt. A number of recent studies have examined links in this chain: ova survival and development (Peterson and Quinn 1996); fry growth and survival (Imre et al. 2005); parr growth (Swansburg et al. 2002; Letcher and Gries 2003; Bacon et al. 2005); and smolt quality (Strothotte et al. 2005). However, to understand the overall productivity of freshwater, we need a synthetic framework within which the interaction of the component processes can be evaluated. The model described in this paper provides such a framework, a particular strength being computational efficiency, which is sufficient for Baysian parameter estimation, and thus rigorous hypothesis testing, to be practical.

In our application of this framework, we caricatured the critical density-dependent processes occurring between adult return and the appearance of catchable fry by an empirical stock (fry) – recruitment curve, which may transfer in form to other arenas but the parameters of which we cannot begin to estimate in such arenas without long-term data comparable with that given in Fig. 3. The clearly positive effect of recent manipulations of ova distribution in the Girnock suggests that even in a water with abundant salmon habitat, changes in ova dispersal or survival may fundamentally affect the processes determining the stock–recruitment relation. Taken together with the expense and difficulty of procuring sufficient data to characterize this clearly noisy process, this demonstrates that empirical characterization of the stock–fry transition is not the optimum route to models that generalize well enough to be useful in a policy context.

We believe that the route to a generalisable and thus widely useful model lies in adding to the broad understanding of the parr growth and smolting process embodied in the model described in this paper a similarly detailed understanding of the dynamical details of the growth and survival of the very earliest stages of the life cycle, between deposition of ova in the redd and the appearance of the fry in conventional late-summer surveys. This will require a focused observational, experimental, and theoretical effort but is, we believe, an achievable target.

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Appendix A. The model

Individuals

Growth

Our model characterises individuals by their length (L) and asserts that in an environment with food abundance (F), this length changes irreversibly at a rate

$$\text{(A1)} \qquad \frac{dL}{dt} = [\alpha\varphi(F) - \mu L]^{+} \text{ where } \varphi(F) = \frac{F}{F + F_H}$$

Here, $[x]^{+}$ implies $\max(x, 0)$, α is a constant of proportionality, μ is the length-specific basal metabolic rate (BMR) (i.e., one-third of the weight-specific BMR), and F_h is the half-saturation food abundance.

The key simplifying assumption is that growth rate variability occurs because food abundance, while constant over periods of time τ (the growth correlation time), varies randomly between individuals in a given growth correlation period and (for a given individual) between successive periods. It is further assumed that for a given individual within a given growth correlation period, the food abundance (F) is either large enough compared with F_h that φ can be approximated as unity, or small enough for φ to be approximated as 0, these two eventualities occurring with probability p and $(1 - p)$, respectively. This makes it possible to define

$$\text{(A2)} \qquad L_\infty = \frac{\alpha}{\mu}$$

to represent the asymptotic length of an individual growing indefinitely in a super-abundance of food and hence rewrite eq. A1 as a probabilistic extension of the von Bertalanffy (1938) model, namely

$$\text{(A3)} \qquad \frac{dL}{dt} = \begin{cases} \mu(L_\infty - L) & \text{with probability } p \\ 0 & \text{with probability } 1 - p \end{cases}$$

Gurney et al. (2007) formally classified individuals using a development index q related to length (L), thus

$$\text{(A4)} \qquad q = -\ln\left[1 - \frac{L}{L_\infty}\right] \quad \Leftrightarrow \quad L = L_\infty[1 - e^{-q}]$$

Some elementary algebra, starting from eq. A3, shows that the rate of change of q, thus defined, is independent of its current value, i.e.,

$$\text{(A5)} \qquad \frac{dq}{dt} = \begin{cases} \mu & \text{with probability } p \\ 0 & \text{with probability } 1 - p \end{cases}$$

This property is vital for an efficient population dynamic description (eq. A14), because it ensures that a representation composed of equal-width development index classes can be updated at time intervals chosen so that all members

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of a given class that survive and grow are in the adjacent class at the next update.

Gurney et al. (2007) showed that with constant values of μ and p, the mean length at age predicted by this model asymptotically approaches L_∞ with a realised von Bertalanffy growth rate

$$\gamma = p\mu \quad \text{(A6)}$$

We shall thus parameterise our model using a realised growth rate γ that affects the behaviour of the mean size at age and a growth probability p that influences the size-at-age dispersion.

Gurney et al. (2007) fitted growth patterns with considerable within-year detail by assuming that the growth probability (p) has a high (summer) value (P) between day (of the year) D_g and day (of the year) D_w and a lower (winter) value, which we write as ωP, otherwise. We follow this assumption but recognise that fry live in a distinct environment from parr, which we recognise by assuming that their summer growth probability is φP. Recalling that river age is measured from 1 January in the year of hatch, we define fry as individuals the river age of which is less than the day of the year on which the fry growing season ends (D_w^f). Hence, if a year contains Y days and $a_y = a \bmod Y$ represents the day of the year on which the cohort has river age a, then we can write the value of p appropriate to an individual aged a as

$$p_a = \begin{cases} \varphi P & \text{if } a < D_w^f \\ P & \text{if } a > D_w^f,\ a_y \geq D_g,\ \text{and } a_y \leq D_w \\ \omega P & \text{otherwise} \end{cases} \quad \text{(A7)}$$

Gurney et al. (2007) fitted data on the growth of single cohorts of trout and salmon parr by regarding L_∞ and μ as constants. However, we wish to understand temperature-driven trends in growth performance, so we need to incorporate temperature effects more explicitly. The energetics of salmonid feeding have been examined by Elliott (1976), who showed that the weight-specific basal metabolic rate of brown trout (*Salmo trutta*) varies exponentially with temperature. Jones et al. (2002) used the same form in an individual-based growth model for juveniles of a different salmonid species (Atlantic salmon). We shall adopt the same exponential formulation but recognise that fry and parr inhabit distinct environments, have a longer growing season, and exhibit density-dependent growth (J. Thorley, FRS-FL, Pitlochry, PH165LB, Scotland, unpublished data). To incorporate density dependence, we assume that the realised growth rate scale for fry has a maximum value η times that for parr and falls linearly as cohort size (N_a) increases. Hence we write realised growth rate of a cohort aged a as

$$\gamma_a = \begin{cases} (\eta\gamma_0 - \xi N_a)\exp[T_a^y/T_\mu] & \text{if } a < D_w^f \\ \gamma_0\exp[T_a^y/T_\mu] & \text{if } a > D_w^f,\ a_y \geq D_g,\ \text{and } a_y \leq D_w \\ \omega\gamma_0\exp[T_a^y/T_\mu] & \text{otherwise} \end{cases} \quad \text{(A8)}$$

where γ_0 is the realised growth rate scale for parr, η is the ratio of zero density growth rate scales for fry and parr, ξ is the density-dependence parameter, T_μ is the characteristic temperature, and T_a^y is the temperature to which cohort y is exposed at age a. For a year of length Y days, T_a^y is related to the river temperature at time t, T_t^R, by

$$T_a^y = T_{Y+a|Y}^R \quad \text{(A9)}$$

In our application of this model to data obtained in the Girnock Burn, we determine T_a^y by linear interpolation of monthly averaged temperature observations at Littlemill, which are assumed to apply exactly at the centre day of the month concerned.

Emigration

Despite considerable observational and theoretical attention, there is still debate about when an individual parr makes the decision to go to sea. It has been suggested (Metcalfe and Thorpe 1992; Thorpe et al. 1998) that an individual that will emigrate in a particular spring run will have changed its growth pattern to accommodate this strategy as early as the previous autumn. However, we have no quantitative evidence for wild Girnock parr that could be used define the parameters for a mechanistic model representing such strategic adjustments, so for simplicity, we assume that individuals that have decided to emigrate will continue to grow normally until they actually leave.

Having taken this view, little if anything further is lost if we simply evaluate the emigration decision at the instant of emigration. Further model simplification can then be achieved by postulating that emigration takes place only at two specific times (i.e., days) of the year, which we denote by D_e^{aut} and D_e^{spr} and refer to as the autumn and spring runs, respectively. When comparing predictions with data, we note that the model is predicting the cumulative emigration up to the nominal run day.

Biological processes normally display considerable individual-to-individual variability, so even in the context of the foregoing simplifications, it seems appropriate to take a probabilistic view of the emigration process. We define ϵ_q^{run} as the probability that an individual with development index q will emigrate at the spring or autumn run and choose

$$\epsilon_q^{run} = \varepsilon^{run}\left(1 + \exp\left[\frac{L_b^{run} - L_q}{L_\infty}\right]\right)^{-1} \quad \text{(A10)}$$

where $run \in [spr, aut]$, as an appropriate functional form, as it varies from zero when L_q is small compared with L_b^{run} up to ε^{run} when L_q is large compared with L_b^{run}, with the width of the zone of transition being determined by L_∞. In line with our decision to regard the size cue for

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emigration as time- and age-independent, we assume that the only difference between autumn and spring runs is the proportion of those large enough to emigrate that actually do so (ε^{run}).

Cohorts and populations

We regard a population as a sequence of cohorts (year classes) each characterised by the year in which its members were hatched and its age a calculated from 1 January in that year. Our description of the cohort state at age a uses a sequence of development index classes of width dq. We use $N^{y}_{q,a}$ to denote the number of individuals in cohort y with development indices in $q \to q + dq$ (that is lengths in $L_q \to L_{q+dq}$, see eq. A4) when the cohort is aged a.

Recruitment

The history of cohort y is deemed to start on day D_{hatch} of year y when R^{y} individuals (the survivors of the eggs deposited by the A^{y-1} females that spawned the previous autumn) emerge from the gravel with mean length L_{hatch} and length variability characterised by coefficient of variation (CV) C_{hatch}. We now need to relate the cohort recruitment R^{y} to the number of spawning adults A^{y-1}.

In Fig. 3, we plot the total number of fry in the Girnock Burn, conventionally estimated from the autumn electrofishing fry survey (Buck and Hay 1984), against the total number of adult females (grilse and multi-sea-winter individuals) spawning in the burn the previous autumn. Survival between spawning and this survey (which normally occurs around day of the year 200) is strongly density-dependent. As the solid line in Fig. 3 shows, we can obtain a satisfactory caricature of this stock–recruitment relation using a shifted logistic function of the form

$$F^{y} = \frac{\gamma^{y} F_0 F_{\max}}{F_0 + \gamma^{y} F_{\max}} \qquad \text{where} \quad \gamma^{y} = \left(1 - \exp\left[-\frac{A^{y-1}}{A_h}\right]\right) \tag{A11}$$

F^{y} is the number of fry at the autumn survey, $F_{\max}$ is the maximum possible number of fry, F_0 is the offset needed to make the curve pass through the origin, and A_h is the population of spawners at which the unshifted logistic function reaches 63% of $F_{\max}$.

Because we have no data that would allow us to test a more complex representation, we arrange that the correct number of fry (F^{y}) arrive at the autumn survey (D_{eff}) by the rather arbitrary device of depositing $R^{y} = F^{y}$ newly hatched individuals into the system on day D_{hatch} and assuming that while they grow according to the requirements of eqs. A7 and A8, they are immortal until they reach the autumn survey at D_{eff}.

To complete our description of this part of the model, we need to assume an explicit form for the length distribution of the newly emerged fry. In the absence of any other information, we assume a lognormal distribution. Hence, our specification of the initial state of cohort y is

$$N^{y}_{q=q_{\text{hatch}}} = R^{y} \int_{L_q}^{L_{q+dq}} \mathcal{N}_{\mathcal{L}}(L_{\text{hatch}}, C_{\text{hatch}})\,dL \tag{A12}$$

where L_q represents the length of an individual with development index q (eq. A4) and $\mathcal{N}_{\mathcal{L}}(a,b)$ represents the probability density function for a log-normal distribution with mean a and coefficient of variation b.

Growth, survival, and emigration

The power of characterising individuals by development index instead of length is that the rate of change of q is the same for all individuals in the cohort (eq. A5). Hence, if we update a cohort with current realised growth rate γ_a and growth probability p_a (which implies a basal metabolic rate $\mu_a = \gamma_a/p_a$) from age a to $a + \tau$, where

$$\tau = \frac{p_a dq}{\gamma_a} \tag{A13}$$

then an individual that has q in $q \to q + dq$ at time t and that does not die or emigrate will be found in $q \to q + dq$ with probability $1 - p_a$ and $q + dq \to q + 2dq$ with probability p_a. Hence, if $S^{y}_{a,\tau}$ is the probability of an individual with development index q surviving from age a to age $a + \tau$ and $E^{a,\tau}_{q}$ is the probability that a similar individual does not emigrate over the same period, then

$$N^{y}_{q,a+\tau} = S_{a,\tau}\left[(1 - p_a)E^{a,\tau}_{q} N^{y}_{q,a} + p_a E^{a,\tau}_{q-dq} N^{y}_{q-dq,a}\right] \tag{A14}$$

Equation A10 defines $\varepsilon^{\text{run}}_{q}$, the emigration probability for an individual that has development index q at the time of a given run. If the spring run takes place on day (of the year) D^{spr}_{τ} and the autumn run takes place on day (of the year) D^{aut}_{τ}, then we can see that the probability of not emigrating between a and $a + \tau$ is

$$E^{a,\tau}_{q} = \begin{cases} 1 - \varepsilon^{\text{spr}}_{q} & \text{if } a_y \le D^{\text{spr}}_{\tau} \text{ and } a_y + \tau \ge D^{\text{spr}}_{\tau} \\ 1 - \varepsilon^{\text{aut}}_{q} & \text{if } a_y \le D^{\text{aut}}_{\tau} \text{ and } a_y + \tau \ge D^{\text{aut}}_{\tau} \\ 1 & \text{otherwise} \end{cases} \tag{A15}$$

Because of systematic uncertainty in the inference of total parr population from electrofishing data discussed above, we shall not test our model against inferred time series of parr abundance. There is thus no reason to model mortality after the autumn fry survey by anything other than a density- and age-independent background rate m. Hence, we write the incremental survival of an individual aged a as

$$S_{a,\tau} = \begin{cases} 1 & \text{if } a \le D_{\text{eff}} \\ e^{-m\tau} & \text{otherwise} \end{cases} \tag{A16}$$

Appendix B. Parameter correlations

The parameters fitted to the individual cohorts 1997–2003 are generally only weakly intercorrelated. There is one exception, which we show in Fig. B1, where we see that the baseline growth rate scale (γ_0) and the summer-to-winter growth rate scale ratio (ω) are strongly correlated. This happens because the data do not define the winter growth halt very precisely so the fit can achieve coincidence with the points measured during the summer with a variety of winter growth rates — higher winter growth implying lower summer growth (i.e., lower γ_0) to match the data.

We see that although all cohorts show the correlation, the 1997, 1998, and 2003 cohorts show the biggest range of γ_0,

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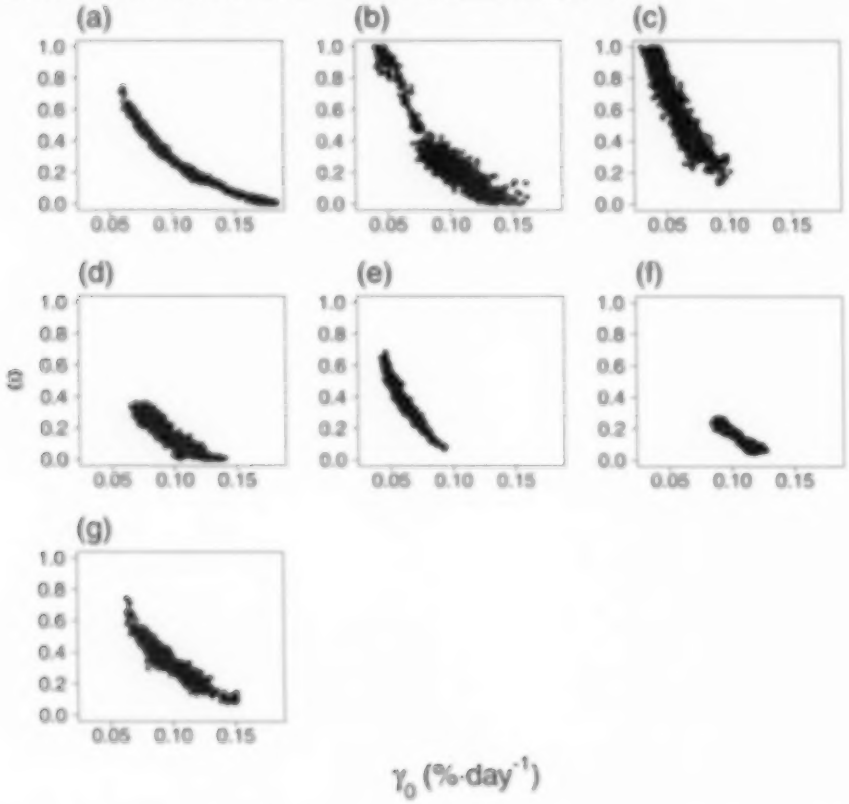
Fig. B1. Correlations between the parr summer realized growth rate scale (γ_0) and the winter-to-summer growth rate scale ratio (ω) in the cohort by cohort fits (*a*) 1997, (*b*) 1998, (*c*) 1999, (*d*) 2000, (*e*) 2001, (*f*) 2002, and (*g*) 2003.



whereas 2002 shows the lowest. This reflects the conformation of the data, particularly with regard to the number and timing of the observations of fry size.

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Continental-scale variation in otolith geochemistry of juvenile American shad (*Alosa sapidissima*)

Benjamin D. Walther and Simon R. Thorrold

Abstract: We assembled a comprehensive atlas of geochemical signatures in juvenile American shad (*Alosa sapidissima*) to discriminate natal river origins on a large spatial scale and at a high spatial resolution. Otoliths and (or) water samples were collected from 20 major spawning rivers from Florida to Quebec and were analyzed for elemental (Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca) and isotope ($^{87}Sr:^{86}Sr$ and $\delta^{18}O$) ratios. We examined correlations between water chemistry and otolith composition for five rivers where both were sampled. While Sr:Ca, Ba:Ca, $^{87}Sr:^{86}Sr$, and $\delta^{18}O$ values in otoliths reflected those ratios in ambient waters, Mg:Ca and Mn:Ca ratios in otoliths varied independently of water chemistry. Geochemical signatures were highly distinct among rivers, with an average classification accuracy of 93% using only those variables where otolith values were accurately predicted from water chemistry data. The study represents the largest assembled database of otolith signatures from the entire native range of a species, encompassing approximately 2700 km of coastline and 19 degrees of latitude and including all major extant spawning populations. This database will allow reliable estimates of natal origins of migrating ocean-phase American shad from the 2004 annual cohort in the future.

Résumé : Nous avons compilé un vaste atlas des signatures géochimiques des jeunes aloses savoureuses (*Alosa sapidissima*) afin de pouvoir reconnaître leur rivière d'origine à la naissance sur une grande échelle géographique et à une forte résolution spatiale. Nous avons récolté des otolithes et (ou) des échantillons d'eau dans 20 rivières importantes pour la reproduction de la Floride au Québec et nous avons mesuré les rapports des éléments (Mg:Ca, Mn:Ca, Sr:Ca et Ba:Ca) et des isotopes ($^{87}Sr:^{86}Sr$ et $\delta^{18}O$). Nous avons examiné les corrélations entre la chimie de l'eau et la composition des otolithes dans cinq rivières où les deux ont été échantillonnées. Alors que les valeurs de Sr:Ca, Ba:Ca, $^{87}Sr:^{86}Sr$ et $\delta^{18}O$ dans les otolithes reflètent les rapports mesurés dans les eaux ambiantes, les rapports Mg:Ca et Mn:Ca dans les otolithes varient indépendamment de la chimie de l'eau. Les signatures géochimiques sont très distinctes d'une rivière à l'autre et la justesse moyenne des classifications est de 93 % lorsqu'on utilise seulement les variables dont la teneur dans les otolithes peut être prédite avec précision à partir des données de chimie des eaux. Notre étude comprend la plus grande banque de signatures d'otolithes provenant de l'ensemble de l'aire de répartition indigène d'une espèce, couvrant environ 2700 km de côtes et 19 degrés de latitude et incluant toutes les principales populations reproductrices actuelles. Cette banque de données permettra dans le futur des déterminations fiables de la rivière d'origine à la naissance des aloses de la cohorte annuelle de 2004 en migration durant leur phase océanique.

[Traduit par la Rédaction]

Introduction

The movements of diadromous fishes during oceanic residency are often poorly understood (Metcalfe et al. 2002; McDowall 2003). Most anadromous species leave their natal rivers to enter marine waters relatively early in their life cycle at a size that is difficult to catch and physically mark in sufficient numbers without inducing major handling effects. Fish may subsequently travel thousands of kilometres before returning to freshwater spawning locations (Dadswell et al. 1987; Quinn and Leggett 1987). Groups of migrating fish collected in oceanic environments often, then, comprise individuals from numerous source populations, though the stock compositions of these mixed groups are generally unknown. Our inability to determine natal origins remains a major problem for determining stock-specific exploitation rates in ocean-intercept fisheries and more generally in attempts to link climate variability with changes in the distribution, growth, and mortality of anadromous fish populations during the ocean phase of their life history.

While conventional tagging studies may be logistically difficult in highly dispersive marine systems, substantial

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progress has been made using natural markers of population affinities (Thorrold et al. 2002). Although rarely as unequivocal as artificial labels, natural tags avoid handling issues and, because all individuals from a population are marked, may be particularly useful when recapture rates are expected to be low. For instance, population genetic markers, including allozyme and DNA loci, have been used for mixed stock analyses of anadromous fishes collected in ocean-intercept fisheries (Brodziak et al. 1992; Brown et al. 1999). More recently, geochemical signatures in fish ear bones (otoliths) have been successfully used to determine natal origins of migratory fishes (Campana and Thorrold 2001). Otoliths are paired aragonitic structures in the inner ears of bony fishes and grow by the continuous accretion of metabolically inert material (Campana and Nielson 1985; Campana 1999). The composition of accreted material reflects the chemistry, temperature, and salinity of the ambient water in which the fish resided (Bath et al. 2000; Walther and Thorrold 2006). Thus, otoliths effectively record variations in environmental parameters over the lifespan of the fish. Fish that are spawned in chemically distinct habitats record these proxies of natal origin in the core region of their otoliths. Each individual from these habitats is indelibly tagged for life, making reconstructions of movement patterns possible (Thorrold et al. 2001).

American shad (*Alosa sapidissima*) are anadromous alosine clupeids native to the east coast of North America. Mature fish spawn in freshwater habitats from Florida to Quebec, beginning in the winter at low latitudes and progressing through the summer at high latitudes (Limburg et al. 2003). Juveniles develop in fresh water and migrate to the ocean, where they spend 3–7 years before returning to fresh water to spawn (Maki et al. 2001). Marine movements of American shad are known from extensive tagging efforts that described complex, long-distance migrations along the North American coast (Dadswell et al. 1987). Despite the potential for substantial stock mixture at sea, most American shad appear to spawn in their natal river (Waters et al. 2000; Walther et al. 2008). There is a pressing need to understand the migratory dynamics of American shad because many populations are under moratorium and stock abundances throughout their range are at historic lows (Olney and Hoenig 2001; Limburg et al. 2003).

Otolith geochemistry has proven a useful natural tag for American shad. Thorrold et al. (1998) demonstrated significant variability of elemental signatures in juvenile otoliths from three major rivers in the northeast United States. Based on a suite of elemental and isotope ratios, Walther et al. (2008) found distinct otolith signatures from 12 natal rivers along the Atlantic coast of the USA. Our present goal was to determine whether distinct signatures could be detected on a continental scale covering the entire native range of the species. We expanded upon the work of Walther et al. (2008) to include all major spawning populations of American shad (ASMFC 2007) across approximately 2700 km of coastline and 19 degrees of latitude. These collections span the entire extant native range of the species. Our second aim was to examine correlations between water composition and otolith elemental and isotope ratios for a subset of these rivers. Where significant, these correlations allowed otolith chemistry to be predicted for rivers where otoliths were not available. Through these analyses, we aimed to create a comprehensive database of signatures for this widely distributed anadromous species, thereby laying the groundwork for future studies to identify natal origins of migrating American shad.

Materials and methods

Otolith analyses

Juvenile American shad were sampled in 2004 from 13 rivers throughout their native range between Florida and Maine (Fig. 1, Table 1). Fish were collected using push nets, seines, and trawls in freshwater and upper-estuarine regions of each river prior to their downstream migration to the oceanic environment. All fish were frozen upon collection. Collections were timed to coincide with highest abundance of juveniles in each river, beginning in January at low latitudes and proceeding through October at higher latitudes. An average of 50 fish (range 18–59) from each river was included in the analyses. Fish were unavailable from the Susquehanna and Chowan rivers, where juvenile abundance was severely reduced in 2004.

Frozen fish were thawed, measured (fork length or total length), and dissected to remove pairs of sagittal otoliths. Otoliths were rinsed in distilled water, air-dried, and mounted on glass slides with cyanoacrylic glue. Mounted otoliths were ground to the midplane using fine-grained lapping film. Ground otoliths were examined for oxytetracycline marks indicating hatchery origin under an ultraviolet (UV) light source. Hatchery-marked juveniles from the Roanoake River were not present in our collections, since they were identified and removed by the North Carolina Wildlife Resources Commission prior to analyses at our laboratory. Hatchery marks were detected in juveniles from the Kennebec (14%, $n = 8$), Exeter (30%, $n = 14$), and Rappahannock (39%, $n = 7$) rivers. Identifying hatchery-marked juveniles for these rivers allowed us to determine whether hatchery fish contained significantly different otolith signatures than wild individuals. After screening for hatchery marks, one otolith from each pair was randomly chosen for laser ablation analysis. This otolith was scrubbed, sonicated in ultrapure water for 2 min, and triple-rinsed in a class-100 clean room.

Cleaned otoliths were analyzed for Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca ratios on a Thermo Finnigan Element2 single collector inductively coupled plasma mass spectrometer (ICP-MS) coupled to a 213 nm laser ablation system. A 200 μm × 200 μm raster was ablated adjacent to the nucleus and extending to the posterior lobe. This raster covered a substantial portion of the juvenile otolith and represented approximately 1 to 3 months of early life. Elemental ratios were quantified by monitoring ^{25}Mg, ^{48}Ca, ^{55}Mn, ^{86}Sr, and ^{138}Ba. Analytical methods followed those of Rosenthal et al. (1999) as modified by Walther et al. (2008). A He gas stream carried ablated material to the ICP-MS, where it was mixed with an Ar sample gas and a wet aerosol (2% HNO_3) supplied by a self-aspirating (20 μm·min^{-1}) PFA nebulizer in the concentric region of the quartz dual inlet spray chamber. Instrument blanks of 2% HNO_3 were run at the beginning, middle, and end of a block of 10 otoliths. A dissolved otolith certified reference material (CRM, Sturgeon et al. 2005) was used to correct for instrument mass bias (Rosenthal et

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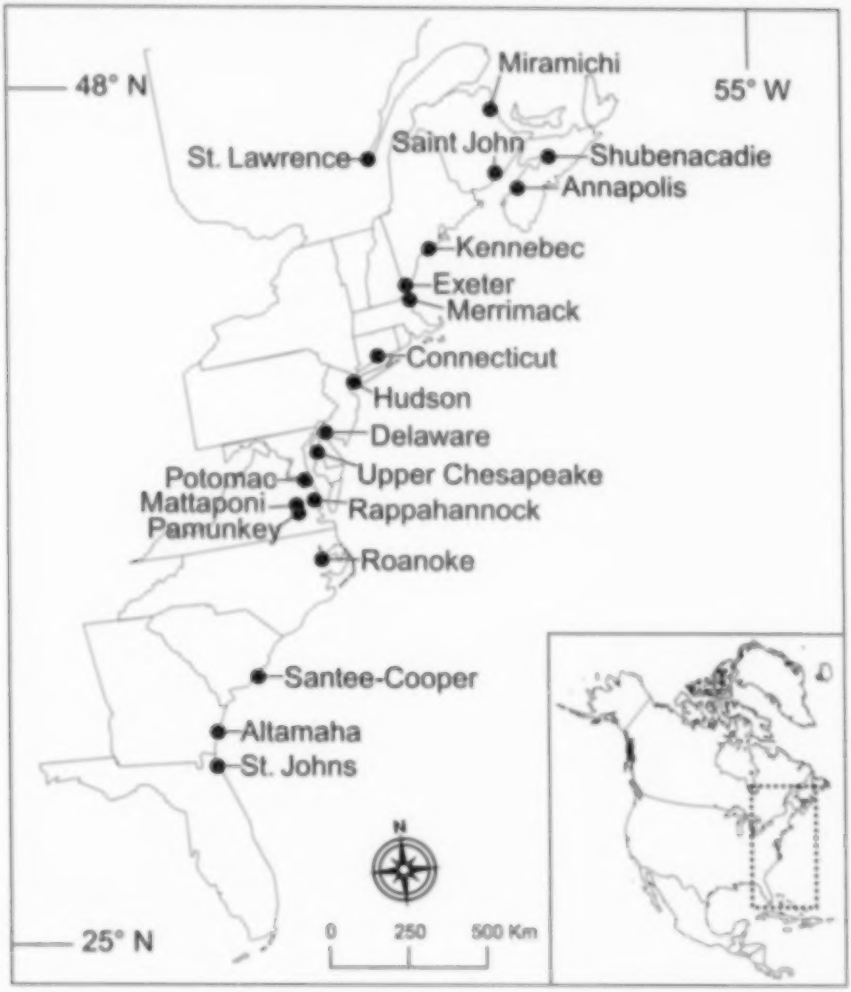
Fig. 1. Map of rivers where otoliths and water samples were collected along the North American Atlantic coast.



al. 1999), and instrument precision was assessed using a second otolith CRM (Yoshinaga et al. 2000). External precisions (relative standard deviations) for the second CRM (n = 134) were 36% for Mg:Ca, 26% for Mn:Ca, 0.3% for Sr:Ca, and 1% for Ba:Ca.

After elemental analysis, the same otolith was used for ^{87}Sr:^{86}Sr ratio analysis using a Thermo Finnigan Neptune multiple collector ICP-MS coupled to a 213 nm laser ablation system. The laser software was used to trace out a 250 μm × 200 μm raster adjacent to the nucleus, extending toward the posterior lobe of each otolith and adjacent to the raster ablated for elemental ratio measurements. Ablated material was carried by a He gas stream from the laser cell to the ICP-MS, where it was mixed with an Ar sample gas and a wet aerosol in a spray chamber as described above for the elemental analyses. In addition to ^{84}Sr, ^{86}Sr, ^{87}Sr, and ^{88}Sr, we monitored ^{83}Kr and ^{85}Rb to account for potential interferences on Sr isotopes (Barnett-Johnson et al. 2005; Jackson and Hart 2006). Following Walther et al. (2008), the contribution of ^{87}Rb to ^{87}Sr intensities was removed by applying a mass bias correction to ^{85}Rb counts derived from measured ^{88}Sr:^{86}Sr ratios. Kr intensities were subtracted from ^{84}Sr intensities until the ^{84}Sr:^{88}Sr value equaled natural abundance ratios (0.006 755), and that Kr value was used to remove ^{86}Kr contributions to ^{86}Sr intensities following Jackson and Hart (2006). All data were normalized to the National Institute of Standards and Technology (NIST) Standard Reference Materials (SRM) 987 ^{87}Sr:^{86}Sr value of 0.71024 based on mean ^{87}Sr:^{86}Sr values measured in SRM 987 for a given analysis day. Mean (±1 standard deviation, SD) values of ^{87}Sr:^{86}Sr values in the SRM 987 (n = 41) and an otolith CRM (n = 74) run throughout the analyses were 0.71025 ± 0.00002 and 0.70916 ± 0.00002, respectively. True values of ^{87}Sr:^{86}Sr are 0.71024 for SRM 987 and 0.70918 for the otolith CRM, both of which are within 1 SD of mean measured ratios.

The second ground otolith from each juvenile was ana-

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Table 1. Juvenile American shad (*Alosa sapidissima*) and water samples collected to groundtruth signatures in each spawning location.

River	Juveniles (*n*)	Length (mm)	Water samples (*n*)
Miramichi	—	—	5
St. Lawrence	—	—	4
Shubenacadie	—	—	4
Saint John	—	—	4
Annapolis	—	—	3
Kennebec	58	52±7	4
Exeter	41	61±8	—
Merrimack	—	—	5
Connecticut	51	58±9	5
Hudson	54	71±9	5
Delaware	57	75±9	—
Upper Chesapeake	58	72±7	—
Potomac	57	52±5	—
Rappahannock	18	60±8	—
Mattaponi	59	58±7	5
Pamunkey	—	—	5
Roanoke	57	81±10	—
Santee-Cooper	57	52±4	—
Altamaha	26	84±6	—
St. Johns	59	78±7	5

Note: Fish lengths (mean ± standard deviation) are total lengths for the Exeter and Roanoke rivers and fork lengths for all remaining rivers.

lyzed for $\delta^{18}O$ values using isotope ratio mass spectrometry (IR-MS). A computer-controlled micromill removed a 400 µm × 400 µm raster with a 75 µm drilling depth. This larger raster incorporated more juvenile material and represented approximately 3 to 4 months of life. However, since all juveniles were captured prior to emigration from fresh water, there was no possibility of incorporating material that represented non-natal habitats. Each milled raster was adjacent to the nucleus and extended toward the posterior lobe. The resulting powder was weighed and transferred to individual glass sample vials. Mean mass (n = 653) of the sampled powder was 46 ± 15 µg (1 SD). Samples were analyzed on a Thermo Finnigan MAT252 equipped with a Kiel III carbonate device following methods outlined by Ostermann and Curry (2000). Isotopic values were reported relative to Vienna Pee Dee Belemnite (VPDB) and expressed in standard δ notation. The long-term precision estimate of the mass spectrometer based on analyses of NBS19 (NIST SRM 8544) is ± 0.07 for $\delta^{18}O$ (Ostermann and Curry 2000).

Water sample analyses

Water samples were collected from 12 rivers along the Atlantic Coast (Fig. 1, Table 1). Samples were taken from three to five locations along the main stem of each river, ranging from just above the salt wedge to the highest region currently accessible by spawning shad, usually determined by a major dam. Collections were made between late August and early October 2004. At each location, 50 mL of river water was collected for Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca, and ^{87}Sr:^{86}Sr ratio analysis. Water was filtered through 0.2 µm Nalgene PTFE (polytetrafluoroethylene) filters with syringes into acid-washed LDPE (low-density polyethylene) bottles and acidified (2%) using concentrated, ultrapure HNO_3. All plastic materials used for sample collection were acid-washed prior to use. An additional 60 mL of river water for $\delta^{18}O$ ratios was filtered into glass vials and sealed with air-tight screw caps. These samples were not acidified. Samples were kept on ice in the field and refrigerated upon return to the laboratory.

Water samples were diluted 10-fold with 2% HNO_3 and analyzed using solution-based ICP-MS to measure ^{24}Mg, ^{43}Ca, ^{55}Mn, ^{88}Sr, and ^{138}Ba on a Thermo Finnigan Element2 single collector as described above. Liquid standards and instrument blanks of 2% HNO_3 were run every six samples. Instrument mass bias was corrected for using certified values of a river water standard (SLRS-4, National Research Council (NRC)), and an internal laboratory river water standard was used to assess measurement precision. External precisions (relative standard deviations) for the laboratory standard (n = 20) were 0.6% for Mg:Ca, 0.5% for Mn:Ca, 0.5% for Sr:Ca, and 0.9% for Ba:Ca.

Prior to analysis of ^{87}Sr:^{86}Sr ratios in river water, a 20 mL aliquot of each sample was evaporated to dryness, redissolved in 50% HNO_3, and passed through columns containing Sr Resin (Eichrom Technologies). This Sr-specific ion exchange resin (4,4′,(5′)-di-t-butylcyclohexano-18-crown-6) separates Sr from other constituents in the water samples via sequential elutions with ultrapure water and 50% ultrapure HNO_3. After Sr separation, the sample was again evaporated to dryness and then redissolved in 1 mL of 5% HNO_3 for Sr isotope analysis using a Thermo Finnigan Neptune multiple collector ICP-MS. Data were corrected for isotopic interferences as described above for otolith analyses. The mean (±1 SD) value of ^{87}Sr:^{86}Sr values in SRM 987 (n = 6) run throughout the analyses was 0.71026 ± 0.00002, which compares favorably with the accepted value of 0.71024 for SRM 987.

The $\delta^{18}O$ ratios of water samples were quantified by equilibrating 0.5 cm^3 of sample with CO_2 at atmospheric pressure following methods described by Epstein and Mayeda (1953). The resulting gas was transferred from the water equilibration system to a Europa GEO 20-20 dual-inlet mass spectrometer. Method precision based on measurements of an internal standard was ±0.08‰ for $\delta^{18}O$ (Swart and Price 2002). Data are reported relative to Vienna Standard Mean Ocean Water (VSMOW) using the standard δ notation.

Statistical analyses

Correlations between water and otolith chemistry were examined using juvenile otoliths and river water samples from the Kennebec, Connecticut, Hudson, Mattaponi, and St. Johns rivers. Linear regressions were fitted to scatterplots of mean ratios of juvenile otoliths against mean river water ratios for all chemical signatures. Mean ratios were used because individual otoliths and water samples were not paired.

Linear regression was used to predict mean otolith values based on mean water sample ratios for those rivers where no juveniles were collected. An estimate of the error about pre-

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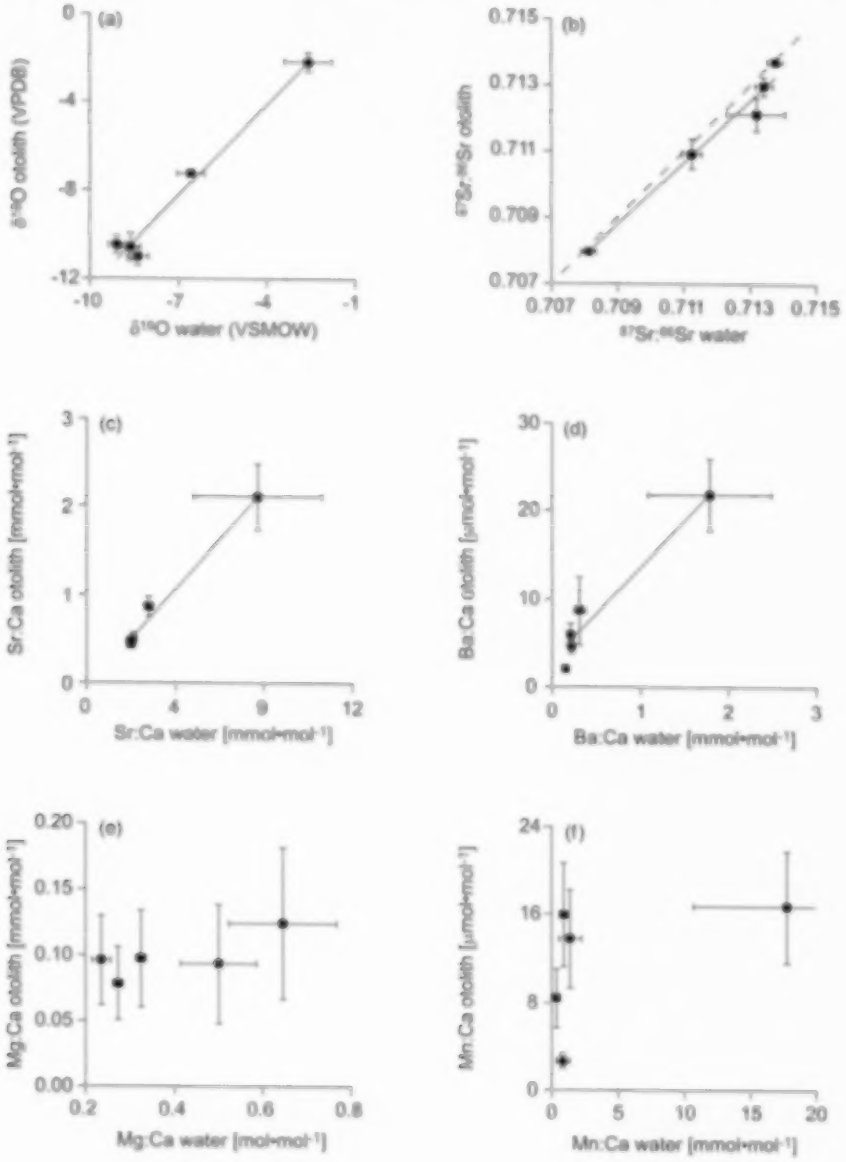
Fig. 2. Regressions of mean ± 1 standard deviation (SD) otolith elemental and isotope ratios against mean water ± 1 SD ratios. Values are for the five rivers where both otoliths and water samples were collected. Regression equations are calculated using mean values. Slopes of $\delta^{18}O$, $^{87}Sr/^{86}Sr$, Sr:Ca, and Ba:Ca ratios are statistically significant. (*a*) $\delta^{18}O$ ratios (VPDB, Vienna Pee Dee Belemnite; VSMOW, Vienna Standard Mean Ocean Water). (*b*) $^{87}Sr/^{86}Sr$ ratios (1:1 relationship is shown as a broken line). (*c*) Sr:Ca ratios, (*d*) Ba:Ca ratios, (*e*) Mg:Ca ratios, (*f*) Mn:Ca ratios.



dicted otolith means was calculated using the equation for the standard error of the prediction error:

$$(1) \quad SE_{pred} = \sqrt{s^2 + s^2_{\bar{y}}}$$

where s^2 is the estimated variance of the regression, and $s^2_{\bar{y}}$ is the variance of the predicted mean otolith ratio for a given mean river ratio (Devore 2004). The standard error of the prediction error was chosen because it accounted for the greater uncertainty inherent in predicting one random variable (an otolith ratio) from another (a water ratio). The exception was $^{87}Sr/^{86}Sr$ ratios, where water values were used as direct predictors of the means

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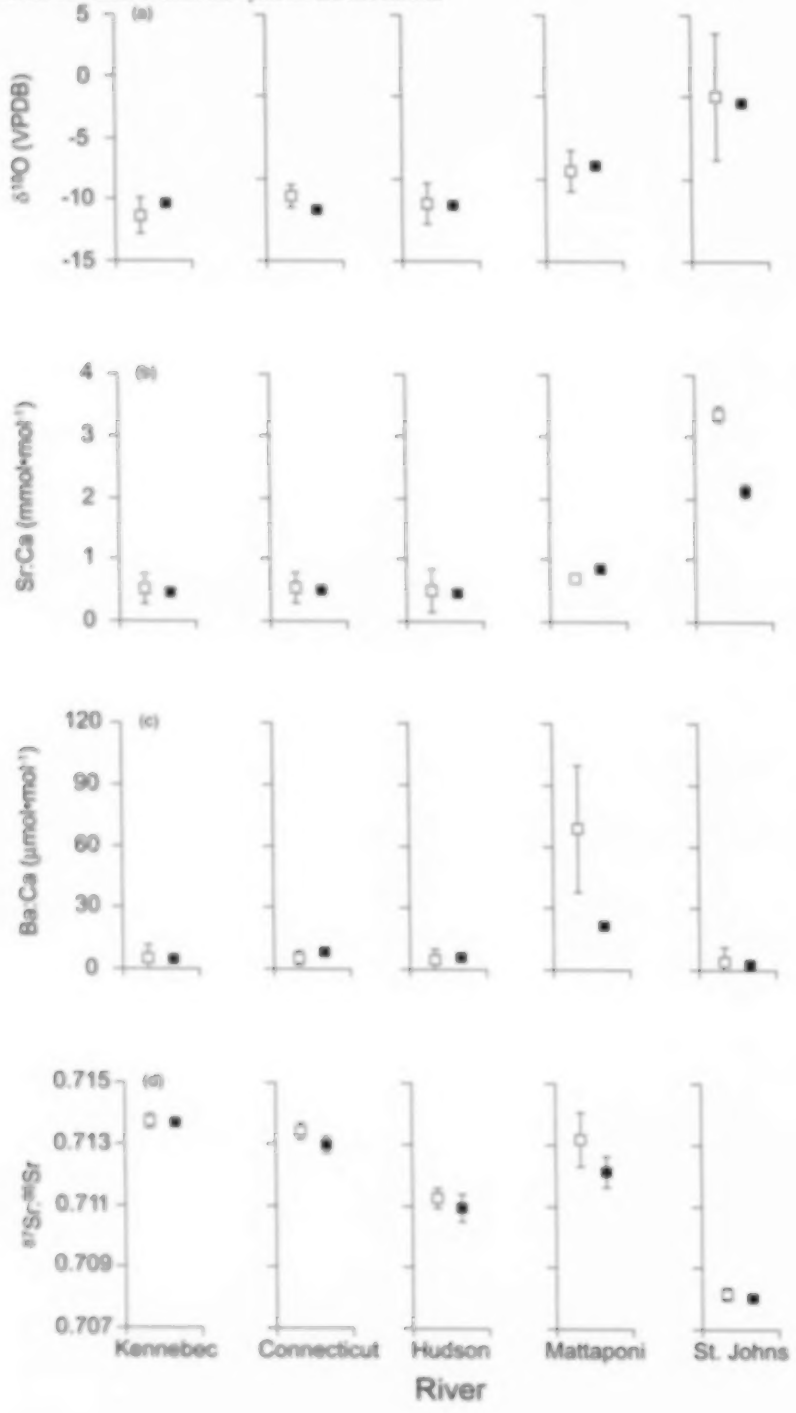
Fig. 3. (*a*) $\delta^{18}O$ values (VPDB, Vienna Pee Dee Belemnite), (*b*) Sr:Ca ratios, and (*c*) Ba:Ca ratios of predicted (open symbols) and actual (solid symbols) otolith signatures for the five rivers where water and otoliths were collected. Rivers were sequentially excluded, and predictions of otolith signatures were based on regressions calculated using the remaining four rivers. (*d*) Comparison of river water (open symbols) and otolith signatures (solid signatures) of $^{87}Sr:^{86}Sr$ ratios. River water $^{87}Sr:^{86}Sr$ ratios and errors were used to directly estimate otolith signatures, so a regression prediction test was not performed as for the other three signatures. For all graphs, means and ± 1 standard deviations (SD) are shown; SDs smaller than the symbols are not shown.



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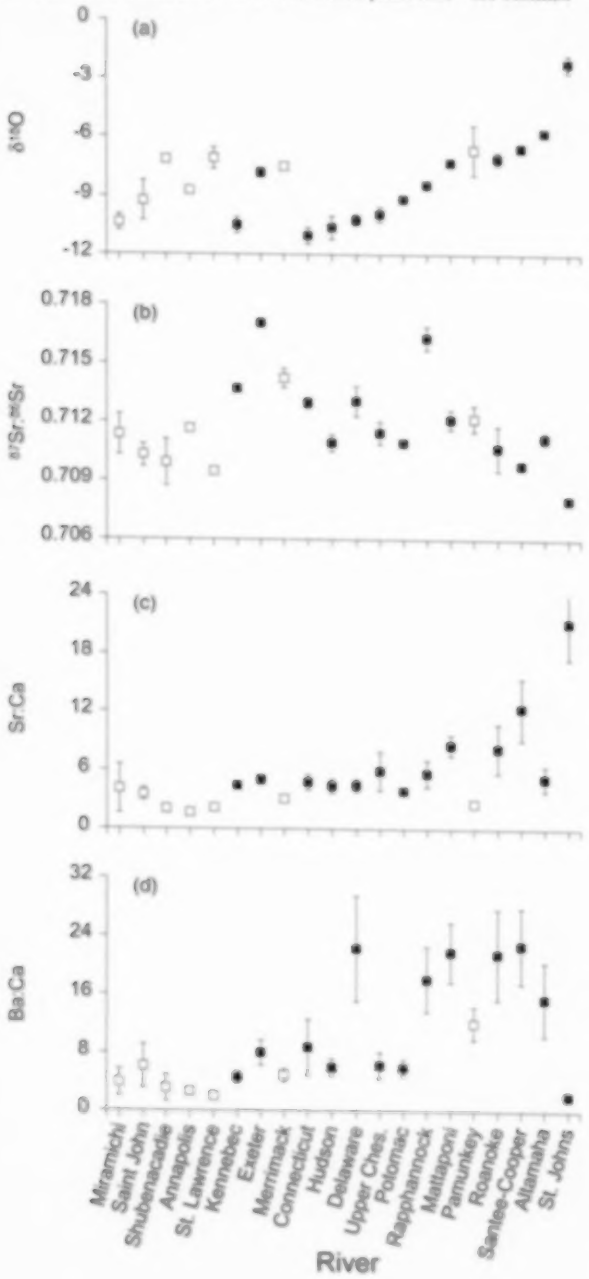

Fig. 4. Elemental and isotope ratios of juvenile otoliths (solid symbols) and water samples (open symbols). Values are means ± 1 standard deviation (SD); SDs smaller than the symbols are not shown. Water samples are displayed for rivers where no juveniles were collected. Rivers are arranged in latitudinal order from north to south along the *x* axis. (*a*) $\delta^{18}O$ values; units are Vienna Pee Dee Belemnite (VPDB) for otoliths, Vienna Standard Mean Ocean Water (VSMOW) for water. (*b*) $^{87}Sr:^{86}Sr$ ratios. (*c*) Sr:Ca ratios; units are mmol·mol^{-1} for water, mmol·mol^{-1} × 10 for otoliths. (*d*) Ba:Ca ratios; units are mmol·mol^{-1} × 10 for water, μmol·mol^{-1} for otoliths.

and standard errors of otolith $^{87}Sr:^{86}Sr$ ratios. We assumed that any deviation from a 1:1 line between $^{87}Sr:^{86}Sr$ values in otoliths and water was due to random errors, because there is no empirical or theoretical support for nonequilibrium deposition of Sr isotopes in fish otoliths (Kennedy et al. 1997, 2000).

We tested the ability of our regression technique to accurately estimate otolith $\delta^{18}O$, Sr:Ca, and Ba:Ca signatures based on water composition using the five rivers where both otolith and water samples were collected. To assess the accuracy of our regression technique for $\delta^{18}O$, Sr:Ca, and Ba:Ca signatures, we sequentially excluded each of the five rivers and recalculated the regression between water and otolith chemistry using the four remaining rivers. We then estimated the mean and standard error of each variable for the excluded river based on the recalculated regressions. We compared the estimated otolith means and standard errors with the observed means and standard errors for each variable in each excluded river. This simultaneously allowed us to assess the influence of individual rivers on the regression equations and determine whether the regression technique adequately constrained predicted otolith ratios.

For each of the seven rivers where no juveniles were collected, 50 fish were simulated with a normal distribution random number algorithm based on predicted otolith ratios and standard errors. A quadratic discriminant function analysis (DFA) was performed on the entire database of simulated and actual otolith signatures from 20 rivers. The DFA used a jackknife cross-validation procedure to determine classification accuracy and distinctness of signatures among rivers. The inclusion of hatchery-marked juveniles did not affect the results of the DFA, and they were therefore pooled with nonhatchery juveniles.

To further confirm that our regression procedure did not bias our predictions of otolith signatures, we compared classification accuracies for simulated and real otoliths for those five rivers used in the regression relationship. First, we restricted our quadratic DFA to only those rivers where juvenile otoliths were present. Classification accuracies for these rivers were close or identical to those obtained with the full database of simulated and actual otolith signatures. Next, we sequentially removed each of the five rivers involved in the regression from the relationship, recalculated the regressions for each chemical signature, and predicted means and errors of otolith compositions for the removed river, as described above. Using those means and errors, we simulated 50 otolith signatures for each of the removed rivers. We then calculated five separate quadratic DFAs, replacing the actual otoliths with simulated otoliths for the excluded river.

Results

Development of a comprehensive, groundtruthed atlas of geochemical signatures in the otoliths of juvenile American shad depended on our ability to reconstruct otolith compositions for rivers where we only collected water samples. We therefore compared water and otolith chemistry from those rivers (Kennebec, Connecticut, Hudson, Mattaponi, and St. Johns) where both juvenile American shad otoliths and water samples were collected. It was possible to predict oto-

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Table 2. Cross-validation summary from the quadratic discriminant function analysis run on signatures from actual and simulated

River	Mira	StLw	Shub	StJn	Anna	Kenne	Exet	Merri
Mira	98 (49)	—	—	2 (1)	—	—	—	—
StLw	—	94 (47)	6 (3)	—	—	—	—	—
Shub	—	14 (7)	80 (40)	—	—	—	—	—
StJn	2 (1)	—	—	98 (49)	—	—	—	—
Anna	—	—	—	—	94 (47)	—	—	—
Kenne	—	—	—	—	—	100 (58)	—	—
Exet	—	—	—	—	—	—	100 (41)	—
Merri	—	—	—	—	—	—	—	100 (50)
Conn	—	—	—	—	—	—	—	2 (1)
Huds	—	2 (1)	—	—	7 (4)	—	—	—
Dela	—	—	—	—	—	—	—	—
UpCh	2 (1)	—	—	—	2 (1)	—	—	—
Poto	—	—	9 (5)	—	—	—	—	—
Rapp	—	—	—	—	—	—	—	—
Matt	—	—	—	—	—	—	—	—
Pamu	—	—	—	—	—	—	—	—
Roan	—	—	2 (1)	—	—	—	—	—
SaCo	—	—	—	—	—	—	—	—
Alta	—	—	—	—	—	—	—	—
StJs	—	—	—	—	—	—	—	—

Note: Reported values are percent classification and numbers of individuals (in parentheses) assigned to each location (indicated by columns). Accurate no classifications. River codes are as follows: Mira, Miramichi River, New Brunswick; StLw, St. Lawrence River, Quebec; Shub, Shubenacadie River, Nova Hampshire; Merri, Merrimack River, Massachusetts; Conn, Connecticut River, Connecticut; Huds, Hudson River, New York; Dela, Delaware River, New Virginia; Pamu, Pamunkey River, Virginia; Roan, Roanoke River, North Carolina; SaCo, Santee-Cooper River, South Carolina; Alta, Altmaha River, Geor-

lith values based on water chemistry data from some, but not all, of the variables that we examined. Four of the six variables showed significant positive linear regressions ($p < 0.01$) between water and otolith chemistry (Fig. 2). The relation between Sr:Ca ratios (mmol·mol^{-1}) in river water ($Sr:Ca_{water}$) and otoliths ($Sr:Ca_{oto}$) was given by

$$(2)\quad Sr:Ca_{oto} = 0.29(\pm 0.02\ SE)\cdot SR:Ca_{water} - 0.08(\pm 0.07\ SE) \qquad (r^2 = 0.99)$$

The relation between Ba:Ca (μmol·mol^{-1}) in water ($Ba:Ca_{water}$) and in otoliths ($Ba:Ca_{oto}$) was given by

$$(3)\quad Ba:Ca_{oto} = 0.01(\pm 0.001\ SE)\cdot Ba:Ca_{water} + 2.80(\pm 1.2\ SE) \qquad (r^2 = 0.94)$$

Otolith $\delta^{18}O$ values ($\delta^{18}O_{oto}$, VPDB) accurately reflected those of the ambient water ($\delta^{18}O_{water}$, VSMOW) according to the following regression:

$$(4)\quad \delta^{18}O_{oto} = 1.4(\pm 0.1\ SE)\cdot \delta^{18}O_{water} + 1.5(\pm 0.9\ SE) \qquad (r^2 = 0.98)$$

The regression between water and otolith $^{87}Sr:^{86}Sr$ ratios deviated only slightly from the 1:1 line:

$$(5)\quad {}^{87}Sr:{}^{86}Sr_{oto} = 0.94(\pm 0.09\ SE)\cdot {}^{87}Sr:{}^{86}Sr_{water} + 0.04(\pm 0.06\ SE) \qquad (r^2 = 0.97)$$

These data confirmed that otolith $^{87}Sr:^{86}Sr$ ratios directly reflect those of the ambient water. However, variations in otolith Mg:Ca and Mn:Ca ($p > 0.05$) showed no detectable dependence on dissolved ratios measured in river water samples (Fig. 2). We therefore excluded Mg:Ca and Mn:Ca from all subsequent analyses.

Our regression technique was generally accurate at estimating mean otolith signatures and conservative in predicting standard errors (Fig. 3). Most predicted mean signatures were within 1 SD of the actual mean. Importantly, predicted otolith SDs were generally at least an order of magnitude larger than the observed SDs. There was, therefore, considerably more variation in water chemistry along a river than was recorded in the juvenile shad otoliths. Presumably this observation was due to the fact that individual juvenile shad were integrating these differences in water chemistry in a similar manner. In any case, our prediction procedure was conservative and provided a much wider range of possible values within a river when compared with observed data in actual otoliths from a given river. This procedure also allowed for expected variability in ambient water chemistry during the juvenile residency period in each river. Predicted and observed Sr:Ca ratios for the St. Johns river were substantially displaced from one another, indicating this river was a high leverage point in the combined regression for this elemental ratio. However, this leverage reflects the true range of ambient Sr:Ca ratios; the St. Johns River is significantly more elevated in Sr:Ca than the remaining four rivers. Similarly, predicted and observed Ba:Ca ratios from the Mattaponi River differed significantly, suggesting that this river also represented a high leverage point for the regression analysis. Again, however, Ba:Ca ratios in the Mattaponi River were higher than those in the other four rivers. When these rivers were removed from the regression calculations for both Sr:Ca and Ba:Ca ratios, the regressions calculated based on the remaining rivers resulted in inaccurate predictions of otolith composition for the Mattaponi and St. Johns

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otoliths (n = 1003, hatchery fish included).

Conn	Huds	Dela	UpCh	Poto	Rapp	Matt	Pamu	Roan	SaCo	Alta	StJs
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	6 (3)	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
—	4 (2)	—	2 (1)	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
90 (46)	—	6 (3)	—	—	—	—	2 (1)	—	—	—	—
—	83 (45)	—	2 (1)	6 (3)	—	—	—	—	—	—	—
—	—	93 (53)	7 (4)	—	—	—	—	—	—	—	—
—	5 (3)	2 (1)	89 (52)	—	—	—	—	—	—	—	—
—	—	—	—	91 (52)	—	—	—	—	—	—	—
—	—	—	—	—	100 (18)	—	—	—	—	—	—
—	—	—	—	—	—	88 (52)	5 (3)	7 (4)	—	—	—
—	—	2 (1)	2 (1)	—	—	6 (3)	86 (43)	4 (2)	—	—	—
—	—	—	—	—	—	2 (1)	—	96 (55)	—	—	—
—	—	—	—	—	—	—	—	2 (1)	98 (56)	—	—
—	—	—	—	—	—	—	4 (1)	8 (2)	—	88 (23)	—
—	—	—	—	—	—	—	—	—	—	—	100 (59)

classifications to group of origin are shown on the diagonal; accuracies sum to 100% across a row for a given source group. Long dashes indicate Scotia; StJn, Saint John River, New Brunswick; Anna, Annapolis River, Nova Scotia; Kenne, Kennebec River, Maine; Exe, Exeter River, New Jersey; UpCh, Upper Chesapeake Bay, Maryland; Poto, Potomac River, Maryland; Rapp, Rappahannock River, Virginia; Matt, Mattaponi River, gia; StJs, St. Johns River, Florida.

rivers. It was therefore important that all five rivers be included in the combined regression for Sr:Ca and Ba:Ca to fully account for the range of possible values across the sampled geographic region.

Elemental and isotope ratios measured in otoliths and water samples varied substantially among rivers (Fig. 4). We found systematic variations in $\delta^{18}O$ values along the latitudinal gradient of individual watersheds, although the pattern was less clear with the inclusion of coastal Canadian rivers. Both Sr:Ca and Ba:Ca were characterized by elevated ratios in many southern rivers, although there was higher within-river variability for both ratios than we observed for $\delta^{18}O$ values. Finally, ^{87}Sr:^{86}Sr ratios showed lower variation among samples within an individual river than the elemental ratios, and with no obvious latitudinal gradient.

We restricted analyses of geochemical signatures among rivers to the four elemental and isotope ratios (Sr:Ca, Ba:Ca, $\delta^{18}O$, and ^{87}Sr:^{86}Sr) for which otolith values could be predicted from water samples. DFA was used to analyze measured or simulated otolith signatures in juvenile American shad collected from a total 20 rivers. Cross-validated classification accuracies ranged from 80% to 100%, with an average rate of 93% (Table 2). Misclassifications were generally restricted to only a few fish per river. Most misclassified juveniles were assigned to rivers in the same geographic region as their natal river. For instance, all seven misclassified juveniles from the Shubenacadie River were assigned to the St. Lawrence River. Mean classification accuracy of simulated otoliths based on water chemistry measurements from rivers where juveniles were not collected (93%) was comparable with the mean classification rate of those rivers where only juvenile otoliths were assayed (96%, Table 3). Importantly, our regression procedure did not substantially bias our estimates of classification accuracies. Classification accuracies were similar regardless of whether actual or simulated otoliths were used for the Kennebec, Connecticut, Hudson, Mattaponi, or St. Johns rivers (Table 3).

Canonical structure coefficients indicated the relative importance of the four variables to the separation in geochemical signatures among rivers (Table 4). The absolute value of the coefficient indicates the relative importance of the ratio in driving combined signature separation along that variate, while the sign describes the direction of the relationship. The first canonical variate was primarily driven by a strong latitudinal cline in $\delta^{18}O$ values, with values becoming isotopically lighter with increasing latitude (Fig. 2*a*). Strontium isotopes and Sr:Ca ratios also contributed to separation among rivers on the first canonical variate. Excluding the Canadian rivers, ^{87}Sr:^{86}Sr ratios typically increased, while Sr:Ca ratios decreased, from low to high latitudes. Loadings on the second canonical variate were dominated by regional variation in ^{87}Sr:^{86}Sr ratios (Fig. 2*b*), with a smaller contribution from $\delta^{18}O$ ratios. Finally, Ba:Ca ratios appeared to contribute minimally to signature separation along the first variate and moderately to separation along the second variate.

Discussion

Geochemical signatures in otoliths and water collected from 20 major spawning rivers along the Atlantic coast of the United States and Canada were excellent natural tags of natal origins in American shad. Cross-validation classification accuracies of individual fish based on these signatures

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Table 3. Cross-validated classification accuracies (%) for quadratic discriminant function analyses (DFAs).

DFA	Kenne	Exet	Conn	Huds	Dela	UpCh	Poto	Rapp	Matt	Rosn	SaCo	Alta	StJs	Average
(*a*)	100	100	94	91	93	93	96	100	92	96	98	92	100	96
(*b*)	**98**	100	92	91	93	93	96	100	92	96	98	92	100	95
(*c*)	95	100	**94**	91	93	93	96	100	92	96	98	92	100	95
(*d*)	100	100	94	**86**	93	95	96	100	92	96	98	92	100	96
(*e*)	100	100	94	91	93	93	96	100	**100**	98	98	92	100	97
(*f*)	100	100	94	91	93	93	96	100	92	96	98	92	**100**	96

Note: (*a*) Classification accuracies for actual otoliths only. All remaining DFAs substituted simulated otoliths for actual otoliths using regressions excluding the simulated river in question: (*b*) Kennebec River, (*c*) Connecticut River, (*d*) Hudson River, (*e*) Mattaponi River, (*f*) St. Johns River. River codes are given in Table 2; bold text indicates the substituted simulated otoliths.

Table 4. Total canonical structure coefficients from the discriminant function analysis performed on actual and simulated otoliths.

Ratio	Variate 1	Variate 2
$\delta^{18}O$	0.887	0.452
Sr:Ca	0.672	0.085
$^{87}Sr:^{86}Sr$	–0.733	0.672
Ba:Ca	0.108	0.182

using DFA averaged 93% and were as high as 100% for five of the rivers. These results agree with previous, more limited work on American shad otolith signatures. Thorrold et al. (1998) were able to distinguish juvenile American shad from the Hudson, Connecticut, and Delaware rivers based on Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca in otoliths. Walther et al. (2008) added C, O, and Sr isotopes to the suite of elements assayed by Thorrold and co-workers and were able to accurately distinguish among juvenile American shad from 12 rivers. We expanded the database to include all major spawning rivers from throughout the native range of American shad, providing a comprehensive assessment of otolith signature variation for this species in 2004. Moreover, our analyses were restricted to variables for which otolith composition could be accurately predicted based on the water chemistry of ambient natal environments. This, in turn, allowed us to groundtruth geochemical signatures in rivers where it was only possible to obtain water samples.

Water chemistry can be used to predict otolith chemistry providing there is a strong correlation between the two variables in natural environments. Experimental work has demonstrated strong effects of ambient Sr and Ba concentrations on otolith composition (e.g., Bath et al. 2000; Elsdon and Gillanders 2003; Kraus and Secor 2004). We also found that Sr:Ca and Ba:Ca in juvenile American shad otoliths were correlated with ambient levels in river waters. In contrast, neither Mg:Ca nor Mn:Ca ratios in otoliths were correlated with dissolved levels in the ambient water in our study. Limited experimental evidence has also suggested that variations in otolith Mn:Ca ratios are not related to dissolved Mn:Ca ratios (Elsdon and Gillanders 2003), while the relationship between water and otolith Mg has not been addressed experimentally. Both Mg and Mn play important roles in metabolic processes yet are toxic at high concentrations (Lehman and Joyce 1993; Rainbow 1997). Fish may regulate Mg and Mn concentrations to physiologically optimum levels, decoupling otolith composition from that of ambient waters. Despite the potential role of metabolism in controlling otolith Mg and Mn, these two elements can still be used successfully as stock identifiers providing there are substantial differences in elemental concentrations among groups of fish. However, because we used water samples to characterize otolith composition where juveniles were unavailable, we could only use elements and isotopes that accrete in otoliths in proportion to their ambient concentrations. Thus, the lack of a significant relationship between otolith and water composition for both Mg:Ca and Mn:Ca ratios in this study means predictions could not be made based on water sample analyses for American shad. For this reason, water samples may not be a reliable substitute for direct analyses of otolith composition for studies that require such elements for habitat-specific signatures. We thus caution investigators to not assume water sampling alone can circumvent the necessary step of groundtruthing spatial signature maps with otolith analyses, particularly for elements that do not directly reflect ambient water composition.

Oxygen isotopes proved to be particularly useful for identifying natal origins of American shad. Otoliths from low latitude rivers recorded isotopically heavy ratios, while otoliths from higher latitudes were progressively less enriched in ^{18}O. Latitudinal patterns in $\delta^{18}O$ ratios of precipitation and surface waters are driven by the Rayleigh distillation process and local mean annual temperatures (Dansgaard 1964; Bowen 1988; Kendall and Coplen 2001). The usefulness of $\delta^{18}O$ ratios for discriminating among rivers was less robust at high latitudes. The slope of the latitudinal cline decreased such that rivers north of the Chesapeake Bay overlap substantially in their $\delta^{18}O$ ratios. This pattern is not surprising because many of the northern rivers drain catchments in overlapping latitudinal regions (Benke and Cushing 2005). Despite this overlap of northern $\delta^{18}O$ ratios, oxygen isotopes were the most important factor driving separation of the multivariate signatures along the first canonical variate. The inclusion of $\delta^{18}O$ ratios therefore contributed substantially to our ability to discriminate among rivers along the large latitudinal gradient surveyed in this study.

Several studies have suggested that Sr isotopes in otoliths may be powerful tracers of fish movements in freshwater environments (e.g., Kennedy et al. 2002; Barnett-Johnson et al. 2005; McCulloch et al. 2005). The current optimism is based on two observations. First, otolith $^{87}Sr:^{86}Sr$ values directly reflect ambient water composition (Kennedy et al. 2000). Second, the geological composition of bedrocks determines the $^{87}Sr:^{86}Sr$ ratio of dissolved Sr in a watershed (Palmer and Edmond 1992). River catchment geology can

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vary over surprisingly small spatial scales, and this in turn generates highly distinctive signatures of individual rivers or streams within a single river watershed (Kennedy et al. 2000). Because the rivers included in this study drained highly heterogeneous surface geologies, river-specific $^{87}Sr:^{86}Sr$ ratios were highly variable and an important component of signature separation. Water samples may thus be generally acceptable substitutes for otolith analyses to characterize habitat-specific $^{87}Sr:^{86}Sr$ signatures on both small and large geographic scales.

Developments in mass spectrometry have also helped to increase interest in otolith Sr isotope ratios. Traditional analysis by thermal ionization mass spectrometry (TIMS) requires that otolith material be dissolved and then passed through a cation exchange resin column before making the isotope measurements on a TIMS instrument. Such a method would have been prohibitively time-consuming to analyze the 650 otoliths in this study. In contrast, laser ablation multiple collector ICP-MS provided a method for in situ Sr isotope analyses with no sample requirement beyond the preparation of a sectioned otolith. Moreover, unlike earlier work (Milton and Chenery 2003), the precision of our measurements were similar to that obtained routinely by TIMS.

Data on habitat-specific geochemical signatures are frequently gathered over limited spatial scales (reviewed in Gillanders et al. 2001). While most efforts have examined variability in geochemical signatures on the order of 100–1000 km, a few investigators included samples from geographically distant locations. Collections of adult Atlantic cod (*Gadus morhua*) otoliths from the east coast of Canada and Iceland were separated by distances of approximately 4000 km (Campana et al. 1994; Campana and Gagné 1995). Proctor et al. (1995) analyzed juvenile southern bluefin tuna (*Thunnus maccoyii*) otoliths from the coasts of South Africa and Australia, separated by up to 10 000 km. Significant differences in elemental signatures in otoliths of northern bluefin tuna (*Thunnus thynnus*) were detected between the Mediterranean Sea and the western Pacific Ocean, over 14 500 km apart (Secor and Zdanowicz 1998). These investigations assessed large-scale signature variability between widely separated and discrete habitats, analogous to the inclusion of an outgroup in genetic analyses. In contrast, variability in geochemical signatures in otoliths among habitats distributed across a large contiguous range has been rarely examined. Our results confirm that the natal origins of anadromous fishes can be determined over large spatial scales and among multiple populations. Moreover, excellent discrimination was achieved with a total of only four variables that could be recovered from either otolith or water samples.

Attempts to identify natal origins of American shad have been made over several decades using a variety of approaches. Early efforts focused on natural variability in meristic and morphometric characters among rivers (e.g., Warfel and Olsen 1947; Carscadden and Leggett 1975; Melvin et al. 1992). Mitochondrial and microsatellite DNA polymorphisms showed subtle but significant variation among fish from groups of rivers throughout the range of the species (Epifanio et al. 1995; Waters et al. 2000; Nolan et al. 2003), and mtDNA polymorphisms have been used to examine the stock structure of oceanic harvests of American shad (Brown et al. 1999). However, most efforts have only detected genetic differences among only a few spawning populations or between pooled populations between regions. Only one study examined mtDNA among sufficient numbers of individual populations to allow mixed stock analyses of oceanic harvests (Brown et al. 1996). However, despite large detectable variation at the population level, success rates for assigning individual fish to natal rivers never exceeded 20% (Brown et al. 1996). A more precise method for determining mixed stock composition of migrating American shad would provide information vital to the effective management of this fully exploited species. Stock abundances are at a fraction of their historic highs (Limburg et al. 2003), with many under moratorium (Olney and Hoenig 2001). While an ocean-intercept fishery is currently closed, any decision to reopen will need to consider the potential mortality that may be exerted on individual stocks. The dynamic and variable marine movements of American shad could be explored effectively by using the groundtruthed database that we have generated to identify natal origins of migrants.

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Interactions between native juvenile burbot (*Lota lota*) and the invasive spinycheek crayfish (*Orconectes limosus*) in a large European lake

Philipp Emanuel Hirsch and Philipp Fischer

Abstract: Freshwater crayfish are successful invaders in many ecosystems and as cryptic nocturnal species display a potential niche overlap with benthic nocturnal fish. In this study, we tested the effects of the invasive spinycheek crayfish (*Orconectes limosus*) on native young-of-the-year (YOY) and adult burbot (*Lota lota*) in Lake Constance. Using mesocosm experiments, we tested if shelter preferences of YOY and adult burbot and crayfish changed between single and mixed species treatments. To further study the role of crayfish as a stressor for burbot, we monitored the nocturnal behaviour of the species in mesocosms using passive integrated transponder (PIT) tag technology and subsequently determined the plasma cortisol levels in burbot after single and mixed species treatments. Spinycheek crayfish successfully repelled YOY burbot from their preferred daytime shelters into alternative, previously unselected shelters. Crayfish also affected the nocturnal behaviour of YOY burbot by eliciting avoidance behaviour and caused an increase in the plasma cortisol levels. While adult burbot did not display any changes between single and mixed species treatments, our results indicate negative effects of spinycheek crayfish on YOY burbot. We conclude that the frequently dense, invasive crayfish populations in lakes may negatively influence local benthic fish populations via their YOY cohorts.

Résumé : Les écrevisses d'eau douce ont envahi avec succès de nombreux écosystèmes et, en tant qu'espèces nocturnes cryptiques, elles peuvent présenter un chevauchement de niche avec les poissons benthiques nocturnes. Dans notre travail, nous évaluons les effets de l'écrevisse américaine (*Orconectes limosus*) envahissante sur les jeunes de l'année (YOY) et les adultes de la lotte (*Lota lota*) indigène du lac de Constance. Des expériences en mésocosme nous ont servi à tester si les choix d'abris des lottes YOY et adultes et des écrevisses changent dans des conditions expérimentales d'isolement ou de cohabitation. Pour étudier plus en détail le rôle des écrevisses comme facteurs de stress pour les lottes, nous avons suivi le comportement nocturne des espèces dans les mésocosmes à l'aide d'une technologie d'étiquettes PIT (à transpondeurs intégrés passifs); nous avons ensuite dosé les teneurs de cortisol plasmatique chez la lotte après des conditions expérimentales d'isolement et de cohabitation. Les écrevisses américaines éliminent avec succès les YOY de leurs abris préférés de jour et les reléguent dans des abris de rechange, non choisis auparavant. Les écrevisses affectent aussi le comportement nocturne des lottes YOY en déclenchant une réaction d'évitement et causent une augmentation des concentrations de cortisol plasmatique. Bien que les lottes adultes ne subissent aucun changement dans les conditions expérimentales d'isolement et de cohabitation, nos résultats indiquent que les écrevisses américaines ont des effets négatifs sur les lottes YOY. En conclusion, les populations souvent denses d'écrevisses envahissantes dans les lacs peuvent influencer négativement les populations locales de poissons benthiques en affectant les cohortes de YOY.

[Traduit par la Rédaction]

Introduction

Crayfish are among the most commonly introduced invasive freshwater species and can have complex effects on aquatic ecosystems (Nyström et al. 1996; Lodge et al. 2000; Bubb et al. 2006). For example, invasive crayfish may lead to a dramatic loss of biodiversity and severe degradation of wetland ecosystems (Rodríguez et al. 2005). Crayfish act as substratum engineers and often affect not just directly competing taxa in a certain habitat but have effects that cascade through the entire ecosystem. Their omnivorous feeding habits can have influences across the trophic spectrum: as predators they target benthic invertebrates, as herbivores they negatively affect macrophytes, and as detritivores they can substantially reduce the organic content of the benthos (Creed 1994; Nyström et al. 1996; Bernot and Turner 2001). Crayfish can form an important or even dominant part of the invertebrate biomass in aquatic ecosystems, with

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population densities up to 315 000 individuals·ha^{-1}. In shallow lakes, annual crayfish production can reach up to 670 kg·ha^{-1}·year^{-1} (Nyström 2002), and in many systems crayfish constitute keystone species (Nyström et al. 1996; Dorn and Mittelbach 1999; Crandall and Buhay 2008). Recent studies have implicated invasive crayfish in the decline of fish populations in rivers, streams (Guan and Wiles 1997; Bryan et al. 2002), and lakes (Covich et al. 1999). Crayfish can alter the availability of suitable spawning substrates (Statzner et al. 2000), directly interfere with breeding fish (Dorn and Mittelbach 2004), and are potential predators of fish eggs or fry (Savino and Miller 1991; Mueller et al. 2003).

For small benthic fish in particular, crayfish may be distinct and even superior habitat competitors, sharing a substantial niche overlap (Griffiths et al. 2004; Carpenter 2005), especially during daytime, when both fish and crayfish often seek for shelter from avian and fish predators (Hill and Lodge 1994; Guan and Wiles 1997; Light 2005).

In Lake Constance, the juvenile burbot (*Lota lota*) and the invasive spinycheek crayfish (*Orconectes limosus*) share a preference for the uppermost littoral zone as daytime habitat, where they seek shelter in the interstices of larger stones and boulders (Fischer and Eckmann 1997; P. Hirsch, personal observation). Burbot are native to many European lake and river systems as well as Lake Constance and, as adults, have some commercial value to local fishermen. Spinycheek crayfish were introduced deliberately into water systems in eastern Germany for commercial fisheries in 1890 and have since spread throughout Central Europe (Schweng 1973; Bohl 1999; Holdich and Black 2007), reaching Lake Constance in the late 1980s. To date, commercial harvests of this species in Lake Constance have remained negligible.

In this study, we focused on interspecific interactions between juvenile burbot and spinycheek crayfish in the shallow littoral zone of Lake Constance. Possible negative effects of the invasive spinycheek crayfish on juvenile burbot were investigated using single and mixed species shelter choice experiments in outdoor mesocosms to elucidate (*i*) whether crayfish and burbot prefer the same stone sizes as daytime habitat and how the two species interact when this resource becomes limited and (*ii*) the behaviour of fish and crayfish when meeting in a shared habitat during their nocturnal foraging phase. Within this experiment, we also determined the circulating plasma cortisol levels in burbot to quantify any possible stress response related to crayfish encounters. All experiments were conducted in outdoor mesocosm setups under light and temperature conditions very similar to those of the natural lake environment.

Materials and methods

Animals

Burbot and crayfish were caught by electric fishing and fyke nets, respectively, during July 2005 in 20–100 cm water depth in the littoral zone of Lake Constance at least 3 weeks prior to the experiments. Burbot could be easily separated in the two age classes: class 0 included specimens with mean total body length (TL) 9.38 cm (±1.4 cm standard deviation (SD)), and class 1 had mean TL 14.9 cm. (±1.76 cm SD). Individuals of each class were maintained in two separate outdoor holding facilities similar to the experimental mesocosms. The crayfish were all of very similar size, with an average carapace length (CL) of 3.46 cm (±0.92 cm SD). They were held together in identical outdoor facilities until the experiments started in August. All holding and experimental facilities were situated approximately 30 m from the littoral area where the animals were caught and were run as flow-through systems supplied with lake water via a 300 µm mesh filter. A surplus of large stones was available in each holding facility to provide all fish and crayfish with sufficient daytime shelter. All animals were fed with frozen chironomids ad libitum every second day. No burbot was used twice for an experiment.

Shelter choice experiments

Single specimen experiments

To assess the shelter preference of solitary fish and crayfish, both species were tested individually in shelter choice experiments. In each experiment, a single specimen (either burbot or crayfish) was placed in a 100 cm × 100 cm square mesocosm test area with a water depth of 20 cm, prepared with a 2 cm deep layer of pebbles. Three additional shelter piles (base dimensions of 25 cm × 25 cm, height 15 cm) were placed in randomized order along one wall of the mesocosm (Fig. 1*a*). Each shelter pile comprised stones of a different size: diameters of about 14.1 cm (±3.0 cm SD), subsequently referred to as large stones; 10.3 cm (±1.5 cm SD), referred to as medium stones; and 4.3 cm (±1.5 cm SD), referred to as small stones. To avoid a bias towards a certain type of stone because of its natural prehistory and to prevent animals recognising previously favoured stones using olfactory cues, the stones were initially obtained from a quarry and were thoroughly cleansed before each experiment using a high-pressure washer.

Each experiment lasted 24 h and started at about noon of the first day, when a single fish or crayfish was selected at random from the holding facility and placed in the experimental mesocosm at the opposite side to the stone piles. Preliminary experiments in identical mesocosms confirmed that both juvenile burbot and crayfish forage mainly during the first half of the night and again around dawn. They then seek a daytime shelter, which they normally do not leave until around sunset (Fischer 2000). For experimental purposes, the daytime shelter preference of the fish and the crayfish was assigned according to their location at noon of the second day. The stone size class of the selected shelter pile was recorded, and CL or TL of the experimental animal was measured to the nearest 1 mm. At least twenty 24 h shelter-choice experiments were performed for each species per size class (burbot age-0, burbot age-1, and crayfish). To test whether animals showed significant preferences for a specific shelter, we tested whether the observed shelter choice significantly differed from a theoretical equal choice of all available shelters using likelihood g^2 tests.

Mixed specimen experiments

To assess competitive interactions between juvenile burbot and crayfish with respect to shelter choice when shelter is limited, the same choice experiments were repeated but

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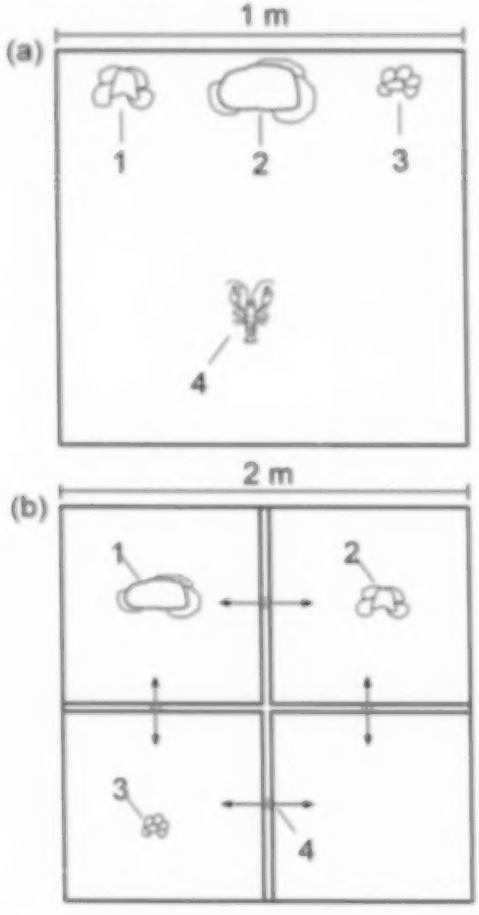
Fig. 1. Schematic picture of the mesocosms used for the different experiments. (*a*) Single and mixed species shelter choice experiments: 1, 2, 3 = large, small, medium shelter piles, respectively; 4 = crayfish. (*b*) Schematic drawing of a passive integrated transponder (PIT) tag mesocosm: 1, 2, 3 = large, small, medium shelter, respectively; 4 = PIT tag gate.



with one burbot and one crayfish placed together in a single mesocosm. As before, all shelter piles were checked at noon of the second day and crayfish CL and burbot TL were measured. Again, at least twenty 24 h experiments were performed for each combination: age-0 burbot with crayfish and age-1 burbot with crayfish. To test whether burbot or crayfish showed significant changes in shelter preference under mixed species conditions, we again used likelihood χ^2 statistic to compare shelter choice in the mixed species treatments with shelter choice in single specimen experiments.

Passive integrated transponder (PIT) tag experiments

To assess the behaviour of fish and crayfish when confronted with each other in a habitat during their nocturnal foraging phase, mesocosm experiments were performed using PIT-tagged animals. All burbot and crayfish used in these experiments were PIT-tagged 3 weeks before the experiments began. In crayfish, PIT tags (TROVAN, 18 mm × 2 mm, glass-encapsulated) were glued on top of the rostrum using two-component adhesive. For this, the rostrum of the crayfish was first cleaned using a sheet of blotting paper dipped in 70% ethanol. The rostrum was roughened with sandpaper and a PIT tag precleaned with 70% ethanol and air-dried was fixed in the groove-like depression at the tip of the rostrum. No crayfish showed signs of adverse reaction to the tagging procedure, and post-tagging mortality was zero for the duration of subsequent experiments. In burbot, the tags were injected into the body cavity as recommended by Fischer et al. (2001). Tagged fish were kept under observation for 25 days after tagging. One out of 20 burbot died of a fungal infection 5 days after tagging (5% after tagging mortality). Otherwise, there was no sign of adverse reaction to tagging and no further after tagging mortality occurred.

The experiments were carried out in two specially equipped outdoor mesocosms (50 cm deep and with base dimensions of 200 cm × 200 cm). The mesocosms were filled with water to a depth of 20 cm and separated by dividers into four identical 100 cm × 100 cm quadrants (Fig. 1*b*). Each quadrant was lined with a 2 cm deep layer of pebbles, and in each of three quadrants, a single pile of either large, medium, or small stones was placed at one wall to provide shelter. At the centre of each divider, level with the substrate, the four quadrants were connected by tube-like passages (2.5 cm diameter). A PIT rod-antenna (diameter 34 mm, TROVAN LN1-639) was mounted in each passage and was triggered by infrared gates continuously registering when a tagged animal, either burbot or crayfish, passed through on its way from one quadrant to another. Previous studies using this system revealed a tag detection rate of 92.5% (Fischer et al. 2001). System control, data storage (date, time, ID), and data compilation (e.g., time of each quadrant change) were carried out by specially designed controlling software. Further data processing was performed by SAS (SAS Institute Inc. 1989). A detailed description of the technical background and data processing protocols is given by Fischer et al. (2001).

As with the shelter choice experiments, the mesocosms were maintained as flow-through systems with the addition of a continuously operating temperature logger (Optic Stow-Away Temp Datalogger, Onset Computer Corporation) located at the centre of each quadrant. On 121 out of 126 days (94% of the duration of the experiment), the temperature difference between the quadrants was less than 1 °C.

Each experiment commenced with the transfer of a single juvenile burbot (of age-0 or age-1) to the mesocosm quadrant without a shelter pile. Data registration began and the whereabouts and diel movement patterns of the fish were recorded continuously. In the afternoon of day three, a tagged crayfish was added to the mesocosm quadrant without a shelter pile, and the movement patterns and quadrant selection of both animals were then recorded simultaneously until day six. During the entire experimental period, food was provided ad libitum daily in the quadrant without shelter pile. At noon of day six, the experiment was stopped and all animals were retrieved from the mesocosm. Six replicates of this procedure were performed for each burbot–

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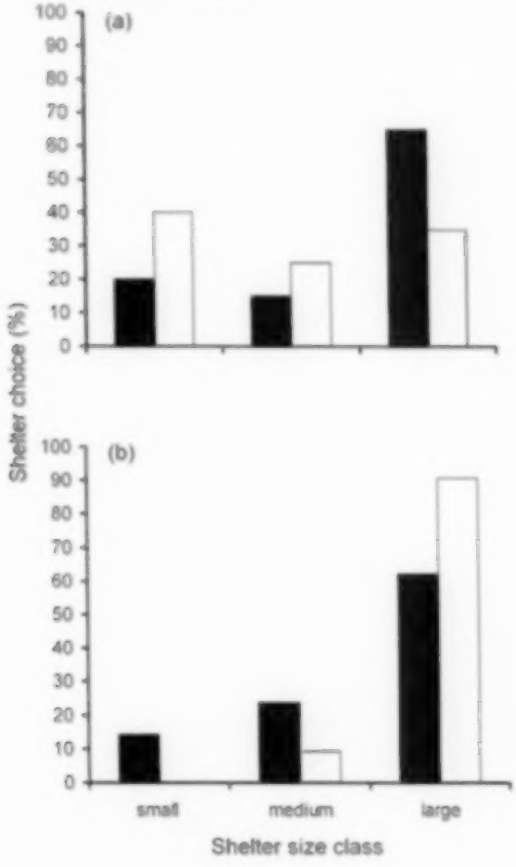
Fig. 2. (*a*) Shelter choice (% of replicates) in age-0 burbot (*Lota lota*) without (solid bars, $n = 20$) and with (open bars, $n = 22$) spinycheek crayfish (*Orconectes limosus*). (*b*) Shelter choice (% of replicates) in age-1 burbot without (solid bars, $n = 20$) and with (open bars, $n = 22$) spinycheek crayfish.

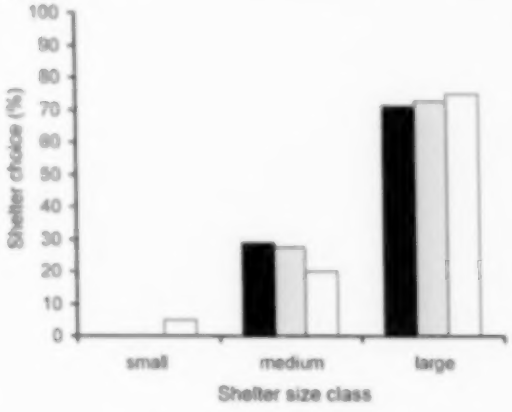


crayfish combination so that a total of 72 nocturnal foraging phases (2 burbot age classes × 6 replicates × 6 days) were recorded. Data relating specifically to occasions where both fish and crayfish entered the same quadrant during nocturnal foraging phases were extracted, and this data set was used to analyse the behavioural response of both animals to each interspecific encounter. Following each encounter, the following alternative scenarios were logged: (*i*) fish leaves the shared quadrant first, or (*ii*) crayfish leaves the shared quadrant first. We defined the first animal to exit a quadrant as the animal retreating. By these data, retreat rates from a shared habitat were calculated for both fish and crayfish. All data were arcsin-transformed prior to further statistical analysis.

Bloodsampling – cortisol enzyme immunoassay (EIA)

To assess physiological stress responses of burbot to the presence of crayfish, blood was sampled nonlethally from the burbot immediately after retrieval from the PIT mesocosm. Fish were transferred directly into a bucket with 5 L of 1 g·L^{-1} TCMP (1,1,1-trichloro-2-methyl-2-2-propanol hemihydrate, CAS No. 6601-64-5) dissolved in lake water. The anaesthetized fish was then punctured into the heart with a 26 G, 0.45 mm × 25.0 mm canula, and 20–100 µL blood was withdrawn into a 1 mL syringe. The entire capture, anaesthesia, and blood-sampling procedure took less than 4.5 min. TL of burbot was also determined (to the nearest 1 mm). The sampled blood was transferred directly to a 100 µL heparinised blood-sampling tube (Microvette, Sarstedt AG and Co., Germany), centrifuged for 3 min at 1400 r·min^{-1} (1 r = 2π rad; 1650*g*) and 4 °C, and the plasma was stored at –80 °C until required for assay. A proprietary EIA kit (Assay Designs Inc., Cortisol Correlate-EIA Kit, Ann Arbor, Michigan) was used to quantify the concentrations of circulating blood plasma cortisol. Plasma samples were diluted 1:30 (in one case 1:60) in assay buffer, and the steroid displacement was used at twice the concentration recommended in the protocol. From then on the kit protocol was strictly followed. The plates were read with a microplate reader (TECAN Rainbow 96 well microplate reader) at 405 nm with correction at 575 nm. Circulating plasma cortisol levels were calculated using the four-parameter, logistic-curve-fitting program of the reader, with the regression coefficient of the standard curve higher than 0.98 in all cases.

Fig. 3. Shelter choice (% of replicates) in spinycheek crayfish (*Orconectes limosus*) alone (solid bars, $n = 20$), with burbot (*Lota lota*) age-0 (open bars, $n = 22$), and with burbot age-1 (grey-shaded bars, $n = 22$).



Results

Shelter choice experiments

In the single species experiments, 75% of age-0 burbot and 61.9% of age-1 burbot showed a significant preference for the largest stone size as daytime habitat (age 0: likelihood χ^2 test; df = 2; $\chi^2 = 8.89$, $p < 0.05$ (Fig. 2*a*); age 1: likelihood χ^2 test; df = 2; $\chi^2 = 5.33$; $p < 0.1$ (Fig. 2*b*)). Crayfish also significantly preferred this habitat type as daytime shelter (likelihood χ^2 test; df = 2; $\chi^2 = 21.44$; $p < 0.05$ (Fig. 3)).

In the mixed species treatments, burbot showed a distinct size-dependent change in shelter choice after crayfish were introduced compared with shelter choice when alone. In age-0 burbot, no more stone-size-specific shelter choice

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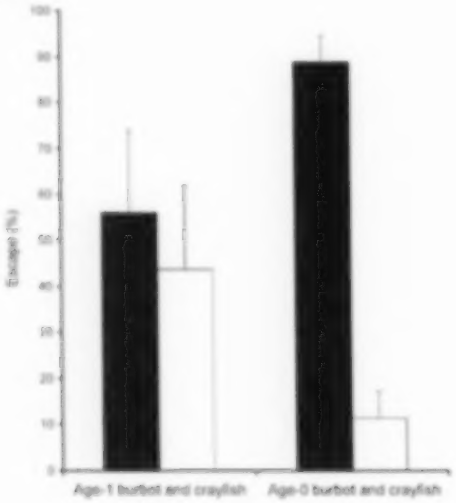
Fig. 4. Percent first-retreats (escapes) of an animal (either spinycheek crayfish (*Orconectes limosus*, open bars), or age-0 or age-1 burbot (*Lota lota*, solid bars)) from a mesocosm quadrant when they met.



could be observed (age-0: likelihood χ^2 test; df = 2; χ^2 = 1.13; not significant (NS) (Fig. 2*a*)), while age-1 burbot began to exhibit an even stronger affiliation for the largest stone size, with more than 98% selection of this shelter type during the day after the introduction of the crayfish (age-1: likelihood χ^2 test; df = 2; χ^2 = 11.25; $p < 0.05$ (Fig. 2*b*)).

In contrast, crayfish shelter preference in the presence of burbot showed no significant difference to that in the single species treatment (with age-0 burbot, likelihood χ^2 test; df = 2; χ^2 = –1.39; NS (Fig. 3); with age-1 burbot, likelihood χ^2 test; df = 2; χ^2 = 0.24; NS).

PIT tag experiments

Both species showed a distinct crepuscular to nocturnal activity pattern. Age-1 burbot started to forage earlier (0.5–1.5 h before sunset) than age-0 (0.5–1.5 h past sunset), and both size classes of burbot ceased their activity about 0.5–1.0 h before sunrise. Similarly, crayfish also started to forage about 1 h prior to sunset and ceased foraging either during the night or around dawn.

Age-0 burbot showed a strong response to the presence of crayfish in the same quadrant during the night. In a total of 1003 of such encounters observed over the entire experimental period, age-0 burbot retreated from the shared quadrant in 88.6% of cases (*U* test; $n = 6$; $m = 6$; $U_s = 55$; $p < 0.01$; Fig. 4), while the crayfish retreated in only 11.4% of cases. In encounters between age-1 burbot and crayfish however, neither species was observed to consistently win out over the other (*U* test; $n = 5$; $m = 5$; $U_s = 32$; NS). In over 531 same-quadrant encounters between age-1 burbot and crayfish, the burbot retreated from the quadrant in 56% of cases, while the crayfish retreated in 44% of cases (Fig. 4). No significant difference was observed in overall nocturnal foraging activity of age-0 and age-1 burbot, based on recorded field changes per hour (*U* test; $n = 15$; $m = 18$; U_s: 215; NS).

Table 1. Plasma cortisol levels for burbot (*Lota lota*) of both age classes in single species treatments (single) and in mixed species treatments (with spinycheek crayfish, *Orconectes limosus*).

	Plasma cortisol level (ng·L^{-1})	
Age class	Single	With crayfish
1+	1.49	1.65
	1.33	1.34
	1.10	1.50
	1.41	1.12
	1.67	1.49
0	2.10	2.25
	1.31	2.07
	1.24	1.51
	1.06	1.44
	1.15	1.69
	1.81	2.13

Level of plasma cortisol

Age-0 burbot responded to the presence of a crayfish in the PIT tag mesocosm with an significant increase in levels of plasma cortisol (Table 1); pairwise *t* test; df = 3; $t = 3.35$; $p < 0.05$). In contrast, age-1 burbot showed no elevated level of plasma cortisol (pairwise *t* test; df = 4; $t = 0.18$; NS).

Discussion

Our experiments revealed distinct size-specific interactions between native burbot and the invasive spinycheek crayfish. In the single species experiments, crayfish as well as both size classes of burbot revealed a significant preference for large stones as daytime shelter. When burbot and crayfish were held together, the larger (age-1) burbot and the crayfish maintained this distinct shelter preference, and both species often (in 12 out of 20 cases) even shared the same shelter pile. In contrast, age-0 burbot significantly changed their primary shelter preference, shifting to an alternative habitat when a crayfish was present. Only 3 out of 20 age-0 burbot were observed sharing large stone piles as shelter with crayfish. Similarly, in the PIT tag mesocosms, only age-0 burbot showed a distinct avoidance reaction to crayfish, and only age-0 fish exhibited a significant increase in plasma cortisol levels following encounters with crayfish.

Invasive crayfish have been well documented as superior competitors for shelter compared with native crayfish species (Söderbäck 1995; Hill and Lodge 1999; Nakata and Goshima 2003), but fewer studies have been made on crayfish–fish interactions. However, Guan and Wiles (1997), Light (2005), and Rahel and Stein (1988) have shown that crayfish may also be superior competitors to fish, capable even of dislodging small benthic fish from their shelters. Our results strongly support these studies and indicate that invasive spinycheek crayfish may have negative effects on sympatric fish communities especially through outcompeting YOY for possibly essential shelter.

For burbot, Fischer (2000) showed that littoral-dwelling

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YOY are especially sensitive to the availability of adequate daytime shelter and that suboptimal shelter may lead to an increase in oxygen consumption of up to 80% because of shelter stress. If such a stress situation persists for an extended period, for example owing to a high spinycheek crayfish population in a certain year, juvenile fish may be forced to compensate for the energy expended in crayfish avoidance behaviour by spending more time in foraging. This is likely to result in an increased exposure to avian and fish predators (Rahel and Stein 1988; Griffiths et al. 2004; Light 2005) and thereby to a higher mortality in the YOY cohort.

Our results also revealed an increase in the level of plasma cortisol in age-0 burbot when a crayfish is present in the same habitat. While the average increase in plasma cortisol levels from 1.42 ng·mL^{-1} (±0.27 ng·mL^{-1} SD) to 1.85 ng·mL^{-1} (±0.35 ng·mL^{-1} SD) observed was significant, it is important to note that plasma cortisol responses to other experimental stresses reported elsewhere have been up to a hundredfold stronger (Svobodová et al. 1999; Jentoft et al. 2005). The difference in amplitude of the response may be explained by the different types of stressors used in our experiments compared with those experiments with much higher poststressor plasma cortisol levels. Most of the available literature on stress responses in fish relates to quite extreme stressor treatments, such as air exposure or extensive manual handling (Pottinger 1998; Pottinger et al. 1999; Grutter and Pankhurst 2000). In contrast, the experimental exposure to a crayfish in the same habitat, as in the procedure outlined above, is certainly a far more subtle treatment, though demonstrably not completely stress-free.

The increases in plasma cortisol levels measured in the experimental fish were significantly higher than those of the control burbot (not having encountered a crayfish) and also significantly higher that those of burbot caught in the wild near the study site (*t* test; df = 11; $t = 3.41$; $p < 0.01$). This and the subtlety of the response lead us to assume that the elevated levels of plasma cortisol observed in our experiments are likely to be a far more realistic representation of in situ competition–stress situations in fish than the extreme elevated levels resulting from the more severe experimental stressors described above.

Our experiments did not allow for a systematic and detailed in situ observation of fish and crayfish behaviour during their nocturnal interactions. However, crayfish were occasionally observed to directly interact with age-0 burbot by actively pinching (using their chelae like pincers) those that came into close proximity. Similar behavioural interactions between crayfish and small benthic fish were described by Griffiths et al. (2004), Guan and Wiles (1997) and Light (2005). In our experiments, burbot reacted to an attack by crayfish with a quick withdrawal swim. This indicates that age-0 burbot perceive crayfish as a major threat, even though they are unlikely to represent a lethal danger. While no mortality of burbot was observed in our experiments, there is evidence elsewhere for direct predation of small fish by crayfish. For example, Guan and Wiles (1997) observed signal crayfish (*Pacifastacus leniusculus*) preying upon small freshwater sculpin (*Cottus gobio*). Breves and Specker (2005) showed that juvenile winter flounder (*Pseudopleuronectes americanus*) reacted substantially to sand shrimp (*Crangon septemspinosa*) attacks, even though these flounder were definitively out of the prey spectrum of sand shrimp. Lima (1998) explained this phenomenon of crude overestimation of a real predation risk, especially in juvenile animals, by the fact that a single miscalculation of the risk posed by a potential predator might be simply irreversible. Our experiments indicate that such cautious reaction towards possible predators is also part of the antipredator behaviour in YOY burbot. Most interesting, however, is the fact that age-1 burbot no longer show this behaviour. In our experiments, age-1 burbot neither retreated nor showed any stress response to the presence of a crayfish in the same habitat, and there were no observed incidents of crayfish aggression towards age-1 burbot. This indicates that (*i*) juvenile burbot are aware of the scope of predation risk relating to their size, (*ii*) spinycheek crayfish are aware of their scope for prey size, and (*iii*) impacts of invasive spinycheek crayfish on native burbot population are most likely to take effect via the YOY cohort.

This indicates that a strong spinycheek crayfish population may have considerable negative effects on the littoral benthic fish community. In Lake Constance, spinycheek crayfish populations are known to fluctuate considerably from year to year, indicating strong interannual population dynamics in this crayfish species (report of local fishermen, Hirsch et al. 2008). Population estimates of crayfish in the area depend on catches with fyke nets that might have a size selectivity towards larger crayfish. However, since our fyke nets caught crayfish down to a carapace length of 1.7 cm and the average size of 547 crayfish caught in the study area was 3.7 cm (±0.3 cm SD) and thus even slightly larger than the crayfish used in our experiments, we assume the used size class as realistic for this one that burbot are most likely to encounter in the lake. High crayfish densities in the littoral zone during a certain year may thus substantially increase competition for shelter not only between crayfish and burbot but also among YOY burbot themselves. For both, juvenile burbot and crayfish shelters are a vital resource (Fischer 2000; Herberholz et al. 2007), and there is evidence that shelters in the wild are limited (Bovbjerg 1970; Hill and Lodge 1994).

Fischer et al. (2004) and Fischer and Öhl (2005) showed that increasing intraspecific competition for shelter in the littoral zone is a most important trigger for juvenile burbot to undergo the obligate littoral–profundal habitat shift by which they finally join the adult population in deep water habitats (Miler and Fischer 2004). According to Hofmann and Fischer (2001), this irreversible habitat shift happens in Lake Constance at a body size of about 14–16 cm TL, but can be temporally shifted towards smaller juvenile fish during periods of high intraspecific competition for shelters (Fischer 2004). A large increase in interspecific shelter competition resulting from high crayfish densities may therefore induce an earlier migration of comparatively small juvenile burbot towards profundal habitats. Because of their small size and the almost complete absence of adequate shelter in profundal habitats, these fish will likely be subject to increased predation, for example by larger and notably cannibalistic adult burbot. Moreover, the stress history of early migrants, for example the potentially long-term increase in plasma cortisol levels caused by high crayfish densities in the littoral zone, may be an additional problem. Such stress

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can lead to a disturbed immune system (Barton et al. 1987; Pickering and Pottinger 1987), impairment of gametes (Campbell et al. 1992; Campbell et al. 1994), and reduced growth (Pickering 1990) (see also review by Wendelaar Bonga (1997)). In combination with the depletion of glycogen reserves in the liver as a result of long-term elevated plasma cortisol levels (Wedemeyer et al. 1990; Begg and Pankhurst 2004), this might lead to an overall poor physiological status in migrating YOY burbot.

It is well known that ontogenetic habitat shifts especially during the juvenile phase of fish are often critical with respect to subsequent recruitment to the adult population (Steele 1997). Beck (1997) concluded that the availability of suitable predator refuges in a critical life phase may constitute a bottleneck for benthic organisms that further limits recruitment. An isochronic occurrence of dense spinycheek crayfish populations and other negative environmental situations such as extreme low-water events during exceptionally dry years can thus contribute to a tightening of the shelter bottleneck for the burbot population. This situation may be of particular future concern since extreme climate and water level events can be expected to increase in both amplitude and frequency. Furthermore, in Lake Constance, as in other European lakes, additional invasive crayfish species like the signal crayfish are either already present or expected as new invaders soon. Therefore, further data on possible interactions between the different crayfish species and native fishes are of the essence if freshwater biodiversity is to be protected as part of our socio-cultural and natural heritage.

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Zooplankton and the total phosphorus – chlorophyll *a* relationship: hierarchical Bayesian analysis of measurement error

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Abstract: Zooplankton grazing is important in resolving residual variation around the total phosphorus – chlorophyll *a* relationship. In empirical studies, zooplankton body size is often a better predictor of residual variation than zooplankton biomass. We investigate whether higher measurement error associated with zooplankton biomass may explain its lower predictive ability. We collected five replicate zooplankton biomass samples in 19 lakes, allowing us to quantify measurement error in volumetric zooplankton biomass with greater precision than in previous studies. A hierarchical Bayesian model was used to assess the predictive ability of volumetric zooplankton biomass and mean individual zooplankton length, corrected for measurement error. We found consistent effects of total zooplankton biomass, but not zooplankton length, on chlorophyll *a*. This finding does not appear to be related to the higher precision with which total zooplankton biomass was measured in our study, but rather to ecological factors. Interlake variation outweighed the effects of measurement error in estimating the strength of relationships between zooplankton variables and chlorophyll *a*. Our findings therefore suggest that studies to estimate zooplankton effects on phytoplankton should allocate resources to study a larger range of lakes over different time periods than to process replicate samples to reduce measurement error.

Résumé : Le broutage du zooplancton est important pour expliquer la variation résiduelle autour de la relation phosphore total – chlorophylle *a*. Dans les études empiriques, la taille corporelle du zooplancton est souvent une meilleure variable prédictive de la variation résiduelle que ne l'est la biomasse zooplanctonique. Nous examinons si l'erreur de mesure plus élevée associée à la biomasse zooplanctonique peut expliquer son pouvoir de prédiction réduit. Nous avons récolté cinq échantillons répétés de biomasse du zooplancton dans 19 lacs, ce qui nous a permis d'évaluer l'erreur de mesure de la biomasse volumétrique du zooplancton avec plus de précision que les études antérieures. Un modèle hiérarchique bayésien a servi à évaluer le pouvoir de prédiction de la biomasse volumétrique et de la longueur individuelle moyenne du zooplancton, une fois l'erreur de mesure corrigée. Nous avons observé des effets convergents de la biomasse du zooplancton total, mais non de la longueur du zooplancton, sur la chlorophylle *a*. Ce résultat ne semble pas relié à la précision plus grande avec laquelle la biomasse du zooplancton total a été mesurée dans notre étude, mais plutôt à des facteurs écologiques. La variation entre les lacs a plus d'effet que les erreurs de mesure sur l'estimation de la force des relations entre les variables zooplanctoniques et la chlorophylle *a*. Nos résultats laissent donc croire que les travaux cherchant à estimer les effets du zooplancton sur le phytoplancton devraient allouer leurs ressources de préférence à l'étude d'une plus grande étendue de lacs à différentes périodes plutôt qu'à l'analyse d'échantillons répétés afin de réduire l'erreur de mesure.

[Traduit par la Rédaction]

Introduction

Models predicting chlorophyll *a* (Chl *a*) from total phosphorus (TP) characterize an important empirical relationship in limnology and underlie current theories of aquatic ecosystem functioning (Sakamoto 1966; Dillon and Rigler 1974). Over orders of magnitude in concentration, TP explains much of the variability in Chl *a*. Nonetheless, the residual variation around this relationship is substantial, and researchers have sought additional variables to help explain differences in Chl *a* among lakes. Zooplankton grazers often explain part of the residual variation, reflecting the effects of grazing and nutrient recycling by grazers on phytoplankton. Although researchers have not always found zooplankton effects, total zooplankton biomass (Currie et al. 1999) or zooplankton body size (length or an index of community size structure) (Pace 1984; Carpenter et al. 1991; Taylor and Carter 1997) explains some of the variation in Chl *a* in most cases. In empirical studies, zooplankton body size is often a better predictor than biomass (Pace 1984; Carpenter et al. 1991; Taylor and Carter 1997).

The superiority of zooplankton body size, versus biomass,

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as a predictor of Chl *a* is puzzling. Most theoretical models of plankton dynamics link biomasses through the functional response equation for grazing; if body sizes enter at all, they affect the parameters of the biomass-based equations. Empirical field studies show that grazing rates depend more strongly on total zooplankton biomass than on body size (Cyr and Pace 1992), and mass-specific grazing rates decline with body size (Peters and Downing 1984). Therefore one would expect biomass to be the better predictor of Chl *a*. Several factors have been proposed to explain strong effects of body size. Greater diet breadth may enable larger zooplankters to consume a wider range of phytoplankton (Gliwicz 1980), but this relationship between diet breadth and zooplankton size depends on taxonomic composition of the zooplankton community (Cyr and Curtis 1999). Larger-bodied zooplankters have higher phosphorus demand and may exacerbate nutrient limitation of phytoplankton through sedimentation or excretion at relatively high N–P ratios (Elser et al. 1988; Sterner and Elser 2002). Finally, the rapid numerical response of large-bodied cladocerans may enable them to keep pace with blooming phytoplankton, at least temporarily (Carpenter et al. 2001). Yet there is another hypothesis — measurement error — that has not been investigated.

Measurement error in predictor variables causes regression analyses to underestimate the slope of the relationship between two variables and thereby underestimate the impact of a predictor that is measured with error (Fig. 1). Measurement error refers to imprecise observation of the true variable of interest. For example, measurement error can be caused by imprecise measuring instruments, by use of surrogate variables to represent the true variables of interest, or from sampling error when, for instance, the true variable of interest is the mean of some ecosystem attribute that is imprecisely estimated. It is well known that measurement error in predictors can have substantial effects on regression results (Fuller 1987; Carroll et al. 2006). Total volumetric zooplankton biomass and mean individual body size are typically measured with different degrees of precision in different studies, and these differences could potentially explain disparate conclusions about the best-fitting model for predicting chlorophyll *a*. We investigate this idea.

The relationship between chlorophyll *a*, phosphorus, and zooplankton is typically analyzed using classic linear regression, an approach that accounts for measurement error in the response variable (Chl *a*) but assumes that variation in predictor variables (e.g., nutrients and zooplankton) is derived from natural processes and that predictor variables are measured without error (Hanson and Peters 1984; Pace 1984; Carpenter et al. 1991). In exploring most ecological relationships, both predictor and response variables are measured with error. If measurement error in predictors is not corrected for in simple linear regression, it leads to attenuation (bias toward zero) of the estimated regression coefficients (Fuller 1987; Carroll et al. 1995; Gustafson 2004) (Fig. 1). The effects of measurement error are worsened in multiple linear regression models. Where two or more correlated predictors are present, and these predictors are measured with different levels of precision, attenuation of the less precisely measured predictor is exaggerated, whereas the coefficient of the more precisely measured predictor becomes inflated (Fuller 1987; Carroll et al. 1995; Gustafson 2004).

In multiple linear regression models of the Chl *a*, TP, zooplankton relationship, TP and zooplankton size and biomass tend to be highly correlated. Additionally, TP and mean individual zooplankton size are measured with relatively high precision (often three replicate TP samples are collected and 20–100 individuals of each species are measured to derive an mean individual zooplankton size estimate per lake (Jeppesen et al. 2000)); total volumetric zooplankton biomass is measured with lower precision (a single composite volumetric estimate is often derived per lake, and at most, triplicate sample are collected) due to the cost of processing many replicate samples (Downing and Rigler 1984; Bird and Prairie 1985). We may therefore expect the TP and zooplankton size coefficients to be inflated, whereas the total zooplankton biomass coefficient may be attenuated. Observed differences in predictive power between zooplankton size and total zooplankton biomass may therefore be a statistical artifact rather than have an ecological basis.

The aim of this study was to investigate whether differences in measurement error could explain the apparent weakness of total volumetric zooplankton biomass, in comparison with mean individual zooplankton size, as a predictor of Chl *a*. Zooplankton biomass may be a weak predictor, relative to mean individual zooplankton length, because zooplankton biomass usually has higher measurement error, relative to zooplankton length, that is not accounted for in classical regression analyses. To explore this idea, we quantified and corrected for measurement error in total volumetric zooplankton biomass and mean individual zooplankton length in a diverse set of lakes. Measurement error can be quantified through replication of field measurements. A unique contribution of this study is that we sampled total volumetric zooplankton biomass more intensively than is customary. Instead of a single tow, or several pooled tows, we collected five full replicate samples from each of 19 lakes. This rigorous replication allowed us to achieve a more precise estimate of measurement error compared with other studies. It follows that the coefficient for zooplankton biomass should be less attenuated when the data are analyzed using a method such as hierarchical Bayesian analysis that can account for measurement error in zooplankton biomass estimates. We used a hierarchical Bayesian model to correct for measurement error in our analyses because of two unique characteristics: (*i*) we were able to expand on a basic linear model to incorporate measurement error associated with predictor variables, and (*ii*) the hierarchical framework allowed for the simultaneous inclusion of variability within lakes and across lakes, as opposed to conventional approaches that focus on the across-lake scale. In our study, because we quantified measurement error in zooplankton biomass with greater precision and accounted for it using a hierarchical Bayesian model, we expected that total volumetric zooplankton biomass would be a stronger predictor of Chl *a* than mean individual zooplankton length.

Materials and methods

Study area

The study lakes are located in the Northern Highlands

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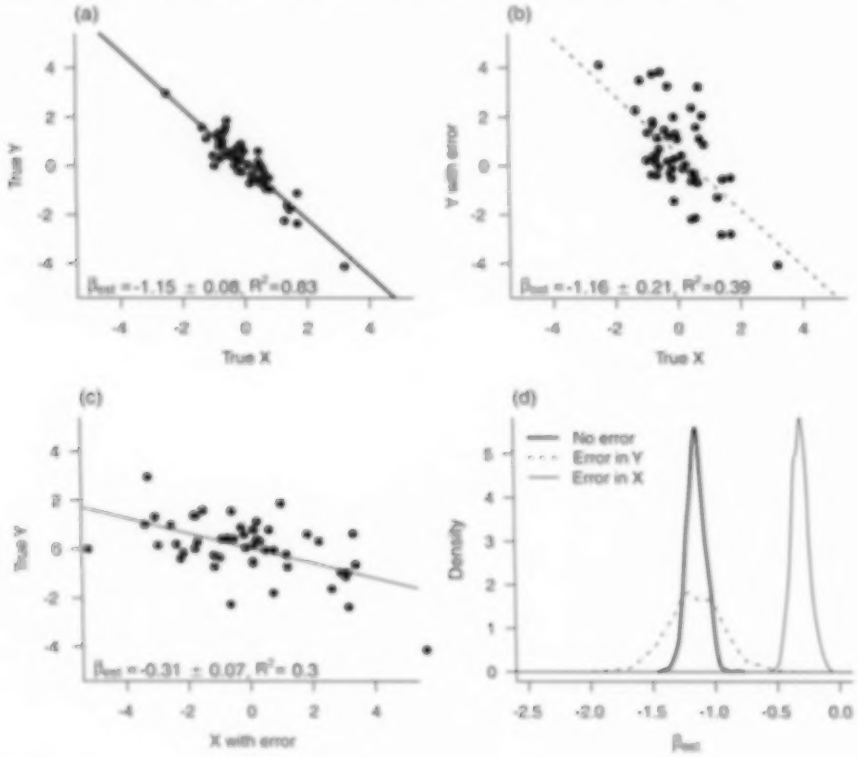
Fig. 1. A simple linear regression model demonstrating the impact of measurement error on the strength of the estimated relationship β_{est} between the true X and Y. Measurement error in the response Y does not substantially affect the magnitude of β_{est}; it simply increases the uncertainty about the estimate (*b* vs. *a*). In contrast, measurement error in X substantially attenuates the strength of β_{est} between X and Y (*c* vs. *a*). Changes in β_{est} are also apparent in histograms of β_{est} derived from a Bayesian analysis: measurement error in Y results in a flattening of the estimated distribution of β_{est}, whereas measurement error in X results in an underestimate of the true strength of the relationship between predictor and response (*d*). Numbers in parentheses indicate two standard errors about β_{est}.



Lake District of Wisconsin, USA. Fourteen of the sample lakes are located in Vilas and Oneida County, Wisconsin, and five lakes lie in Gogebic County, Michigan, within the University of Notre Dame Environmental Research Center (Fig. 2). Lakes were chosen to represent a range of zooplankton sizes and chlorophyll *a* and total phosphorus concentrations. All lakes were sampled once between 30 May and 16 June of 2006 at central, deep-water locations.

Sampling

Chl *a* concentration was determined as a measurement of algal biomass. From each lake, six integrated samples were collected: three from the surface to the bottom of the epilimnion and three from the surface to the bottom of the metalimnion. Integrated samples were mixed in large opaque bottles and filtered in the field through glass fiber filters under low pressure using a peristaltic pump and inline filtration. Filters were frozen and later extracted in Optima methanol before fluorometric analysis according to US Environmental Protection Agency standard methods (Holm-Hansen and Riemann 1978; Arar et al. 1997). Measures were taken at each phase of Chl *a* analysis to avoid exposure to light. Final pigment concentrations were corrected for the influence of phaeopigments.

Whole-water samples for determination of TP were collected in acid-washed polyethylene bottles. Triplicate integrated samples were collected from the epilimnion. Discrete TP samples were collected from the middle of the metalimnion, the top of the hypolimnion, and 1 m above the bottom of the lake using a peristaltic pump. Samples were kept on ice until return to the lab and then preserved using Optima HCl. TP concentrations were analyzed using a Technicon Autoanalyzer following persulfate digestion (American Public Health Association, American Water Works Association, and Water Pollution Control Federation 1995).

We collected five zooplankton samples from each lake using vertical net hauls of a standard Wisconsin net with an anterior reducing cone (80 μm Nitex mesh, 0.22 m diameter mouth). The net was lowered to 2–5 m above the lake bottom and raised at a speed of approximately 0.33 $m \cdot s^{-1}$. Zooplankton were anesthetized and preserved in 70% ethanol. Assuming 100% efficiency of a Schindler–Patalas trap, the net efficiency of the Wisconsin net was determined to be approximately 66% based on samples from a single lake. We did not correct our volumetric zooplankton biomass values for net efficiency. If net efficiency is constant among lakes, it has no effect on variances of linear regression models.

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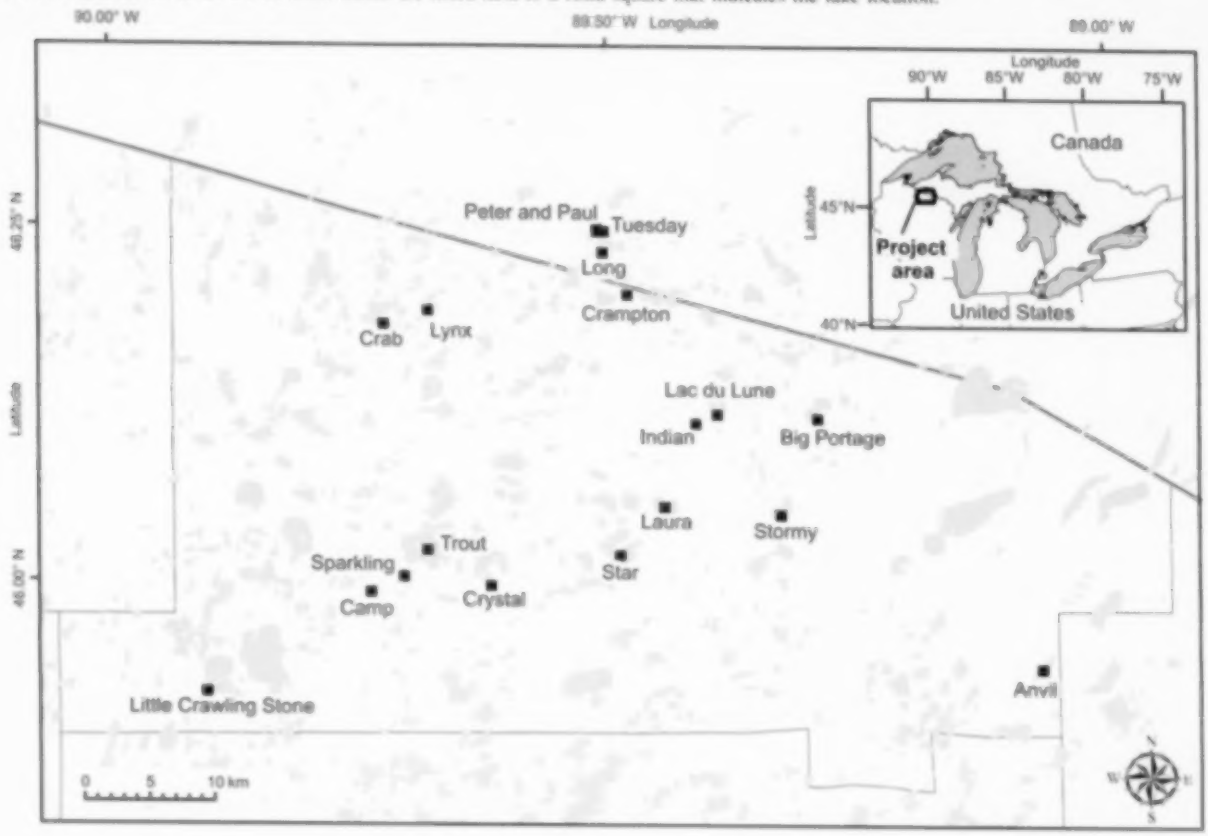
Fig. 2. Map of lakes sampled in the Northern Highlands Lake District of Wisconsin, USA. All lakes lie within the coordinates 45°36′ to 46°18′N and 89°00′ to 89°54′W. Lake names are listed next to a solid square that indicates the lake location.



However, we did not test this assumption by measuring efficiencies in each individual lake.

Zooplankton counting and biomass estimation

Each tow sample was drained through an 80 µm mesh to remove all ethanol and then reconstituted to 50 mL with Milli-Q. The 50 mL sample was subsampled three times with a 1 mL Hensen–Stempel pipette. All crustacean zooplankton in each 1 mL subsample were counted, and up to 30 of each species or genus were measured to obtain species specific mean lengths. Total length measurements (excluding caudal rami and setae) were recorded for all copepods. All cladocerans except *Holopedium gibberum* were measured for total length of the carapace to the base of the spine. *Holopedium gibberum* lacks a rigid carapace, so the distance between setae natatores and the terminal claw of the postabdomen was chosen for measurement, as this part of the animal is more rigid and better represents the size of preserved specimens (Persson and Ekbohm 1980). The terminal claw of *H. gibberum* was measured at 50× magnification to the nearest 0.02 mm, and all other zooplankton were measured at 25× magnification to the nearest 0.04 mm using an ocular micrometer on a dissecting microscope.

All crustacean zooplankton were identified to genus, and in some cases, specimens were identified to the species level. The genera identified include most *Daphnia* spp., *Bosmina* spp., *Ceriodaphnia* spp., *Chydorus* spp., *Diaptomus* spp., *Alona* spp., and *Acanthocyclops* spp. Those identified to species include *Daphnia pulex*, *Daphnia retrocurva*, *Diacyclops thomasi*, *Diaphanosoma birgei*, *Epischura lacustris*, *Eucyclops agilis*, *Holopedium gibberum*, *Leptodora kindtii*, *Mesocyclops edax*, and *Tropocyclops prasinus*. All copepod nauplii and copepodites that were too early in development to have defining adult characteristics were identified to the genus level.

Mean individual zooplankton length (ZL) for each lake was calculated by taking the weighted mean lengths of each species or genus across a sample and then averaging among the five samples to determine the mean individual zooplankton length within each lake. To estimate weight, mean lengths for each species or genus in each of the five samples were converted to mean dry weight estimates using published length – dry weight regressions (O'Brien and de-Noyelles 1974; Dumont et al. 1975; Bottrell et al. 1976; Culver et al. 1985; Lawrence et al. 1987; Yan and Mackie 1987) (Appendix A, Table A1). Biomass for each genus or species was calculated by multiplying the density per litre by the estimated mean weight per individual. Genus- or species-specific biomass estimates were summed to get a volumetric estimate of biomass for each sample, and then

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the five samples were averaged to get a grand mean biomass per lake. Therefore, zooplankton biomass (ZB) represents the total crustacean zooplankton biomass per volume of lake water (μg dry weight·L^{-1}).

Data analysis

We used a hierarchical Bayesian modeling framework to assess the combined effects of TP, mean individual ZL, and total volumetric ZB on Chl *a* concentrations. We chose to use a hierarchical Bayesian framework as it allowed us to explicitly model the error in ZL and ZB measurements within each lake and account for this uncertainty in the estimated model parameters (Ellison 2004; Clark 2005). We carried out the analyses using the R2WinBUGS package (Sturtz et al. 2005) within the statistical software R (R Development Core Team 2007). (Code for the model can be found in Supplemental Appendix S1, available online from the NRC Data Depository.)[1]

The central process and parameters in which we were interested are given by the following linear model, based on observations from each lake (i):

$$(1) \quad \log(\text{Chl}a)_i = \beta_0 + \beta_1 \log(\text{TP})_i + \beta_2 \text{ZB}_i^T + \beta_3 \text{ZL}_i^T + \varepsilon_i$$

The Chl *a* and TP values in this model were taken as the average log-transformed concentrations in the epilimnion and metalimnion combined because this depth range encompassed the photic zone and best represented the characteristics of the water column over which the zooplankton tow was hauled. The average concentrations were calculated from the replicate measurements of Chl *a* and TP in each lake, and we used geometric means because the data were log-normally distributed. We assumed that these mean values represented the true mean Chl *a* and TP in each lake, measured with negligible error. The residual errors ε_i were assumed to be $n(0, \sigma^2)$ distributed.

We explicitly modeled the measurement errors in ZL and ZB in each lake based on the five replicate tows (j) of the water column, assuming an additive measurement error model. For instance, for ZB, we assumed that the observed value in a particular tow ZB_{ij} reflected the true mean ZB_i^T plus measurement error $u_i \sim n(0, v_i^2)$ in the particular lake:

$$\text{ZB}_{ij} = \text{ZB}_i^T + u_i$$

An analogous relationship was assumed for ZL. We modeled the measurement error in each lake separately because it varied substantially among lakes. We assessed the impact of correcting for measurement error by comparing the parameter estimates of a model based on a single tow with parameter estimates based on a model in which compilation of all five tows allowed us to estimate and correct for measurement error.

We used noninformative $n(0, 100^2)$ priors for the regression parameters β_i and noninformative uniform densities $u(0, 100)$ for the standard deviation parameters σ and v_i. As recommended by Gelman (2006) and Gelman and Hill (2007), we did not use inverse-gamma(α, α) prior distributions for the standard deviations as the resulting inferences are sensitive to α when the standard deviations are close to zero.

Results

Surveyed lakes covered a reasonable range in physical, chemical, and biological characteristics. Chl *a* concentration varied between 1.05 and 13.59 μg·L^{-1} (Table 1). TP and total nitrogen (TN) concentrations in the 19 lakes, averaged over the epilimnion and metalimnion, ranged from 7.6 to 21.6 μg·L^{-1} (Table 1) and from 223.1 to 493.6 μg·L^{-1}, respectively. Thus, the lakes sampled spanned a gradient from oligotrophic to mesotrophic. Morphometries varied between the study lakes, with surface areas ranging from 1 to 1608 ha and maximum depths ranging from 7.9 to 35.7 m (Table 1). Morphometric characteristics were not related to TP or Chl *a* concentrations (Pearson's correlation, $p > 0.10$ for Chl *a* and surface area, Chl *a* and maximum depth, TP and surface area, and TP and maximum depth). Although all of the lakes were deep enough to stratify, some were not completely stratified when samples were collected. There was no significant difference in mean Chl *a*, TP, ZL, or ZB between groups of lakes that were or were not stratified (Student's t test, $p > 0.3$ in all cases).

We recorded 17 different genera or species of crustacean zooplankton in the 19 lakes. *Bosmina* spp., *Daphnia* spp., *Diacyclops thomasi*, *Diaptomus* spp., *Holopedium gibberum*, copepodites, and copepod nauplii most commonly dominated the zooplankton communities. In eight lakes, cladoceran biomass accounted for 50% or more of the total biomass, and 18 lakes had at least one *Daphnia* species present (Fig. 3). *Daphnia pulicaria*, the largest herbivorous zooplankton in these systems, were found in seven lakes and contributed significantly to the biomass estimates of Big Portage, Lake Laura, and Star Lake (52%, 73%, and 61%, respectively). At the other end of the spectrum, cladocerans made up less than 20% of the biomass in five lakes. The remaining six lakes had mixed crustacean zooplankton communities, including cyclopoid and calanoid copepods and cladocerans. Mean ZB varied considerably among lakes, ranging from 2.2 to 438.1 μg dry weight·L^{-1}, whereas mean length of the zooplankton community per lake ranged from 0.27 to 0.74 mm (Table 1). Taken together, these results indicate that sampled lakes represent a wide range in zooplankton biomass and community size structure.

We recorded substantial variability in Chl *a*, TP, ZB, and ZL both within and among lakes, as reported by the coefficient of variation (CV) in Table 1. The CV based on replicate measurements in each lake provides an estimate of the measurement error associated with each variable. It is notable that the CV, and hence estimated measurement errors, varied considerably from lake to lake. The range of CVs was smallest for ZL (1.2%–11.3%), followed by Chl *a* (1.1%–17.8%, except Anvil Lake (46%), which was identified as an outlier using Grubb's test). The CV ranges for TP and ZB were relatively large (2%–26% and 4%–35%, respectively). The CV of ZB was related to tow depth

[1] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 3858. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/cms/unpub_e.html.

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Table 1. Morphometric characteristics and mean values of limnological variables for 19 lakes in Northern Wisconsin.

Lake	Surface area (ha)	Maximum depth (m)	Tow depth (m)	Chl *a*[a] ($\mu g \cdot L^{-1}$)	CV Chl *a*	TP[b] ($\mu g \cdot L^{-1}$)	CV (TP)[c]	ZL[d] (mm)	CV (ZL)	ZB[e] (μg dw$\cdot L^{-1}$)	CV (ZB)
Anvil	154	9.8	6.0	4.05	0.467	16.47	0.021	0.38	0.113	116.24	0.354
Big Portage	258	12.2	7.0	1.84	0.051	10.93	0.208	0.44	0.108	148.47	0.305
Camp	15	9.4	5.5	1.73	0.051	9.72	0.049	0.27	0.035	25.63	0.109
Crab	384	18.3	13.0	5.53	0.173	12.63	0.169	0.51	0.025	114.22	0.058
Crampton	26	20.0	14.0	4.56	0.064	12.06	0.026	0.57	0.069	89.21	0.041
Crystal	37	20.4	15.0	1.48	0.043	9.57	0.105	0.54	0.058	162.14	0.159
Indian	161	7.9	5.0	2.59	0.039	17.29	0.090	0.52	0.074	278.98	0.187
Lac du Lune	172	20.7	14.0	1.82	0.020	9.88	0.019	0.47	0.022	185.32	0.096
Lake Laura	242	13.1	8.0	1.98	0.071	10.99	0.139	0.74	0.037	128.86	0.220
Little Crawling Stone	43	13.4	10.0	4.28	0.025	15.12	0.091	0.41	0.029	154.60	0.079
Long Lake	6	17.7	14.0	11.57	0.045	21.64	0.099	0.34	0.069	6.33	0.261
Lynx Lake	137	14.6	9.0	3.14	0.011	9.28	0.115	0.55	0.040	124.35	0.153
Paul	2	15.0	9.0	8.46	0.144	21.14	0.142	0.37	0.059	27.87	0.120
Peter	3	19.3	14.0	12.16	0.093	15.17	0.188	0.33	0.030	23.53	0.142
Sparkling	64	20.0	15.0	4.96	0.041	9.50	0.074	0.44	0.012	105.74	0.036
Star	465	20.4	16.0	1.05	0.050	7.59	0.083	0.60	0.062	177.51	0.076
Stormy	211	19.2	14.0	1.21	0.071	8.25	0.260	0.50	0.027	438.06	0.073
Trout	1608	35.7	30.0	2.16	0.019	12.91	0.151	0.50	0.025	122.89	0.079
Tuesday	1	16.5	12.0	13.59	0.037	21.03	0.159	0.29	0.017	2.18	0.136
Average	210	17.0	12.1	4.64	0.080	13.22	0.115	0.46	0.048	128.01	0.141
CV among lakes				0.849		0.340		0.254		0.806	

[a]Chl *a* values represent the epilimnion + metalimnion.
[b]TP (total phosphorus) values represent the epilimnion + metalimnion.
[c]CV (TP) values are based on replicate samples in the epilimnion only. CV, coefficient of variation.
[d]Zooplankton length (ZL) is the weighted mean length per sample, averaged across replicates.
[e]Zooplankton biomass (ZB) is reported in dry weight (dw).

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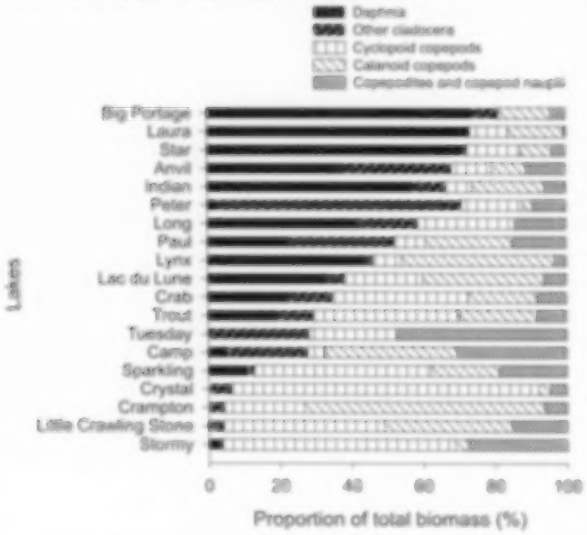
Fig. 3. Macrozooplankton community composition for 19 lakes in northern Wisconsin. The lakes are arranged vertically according to the relative abundance of cladoceran zooplankton.

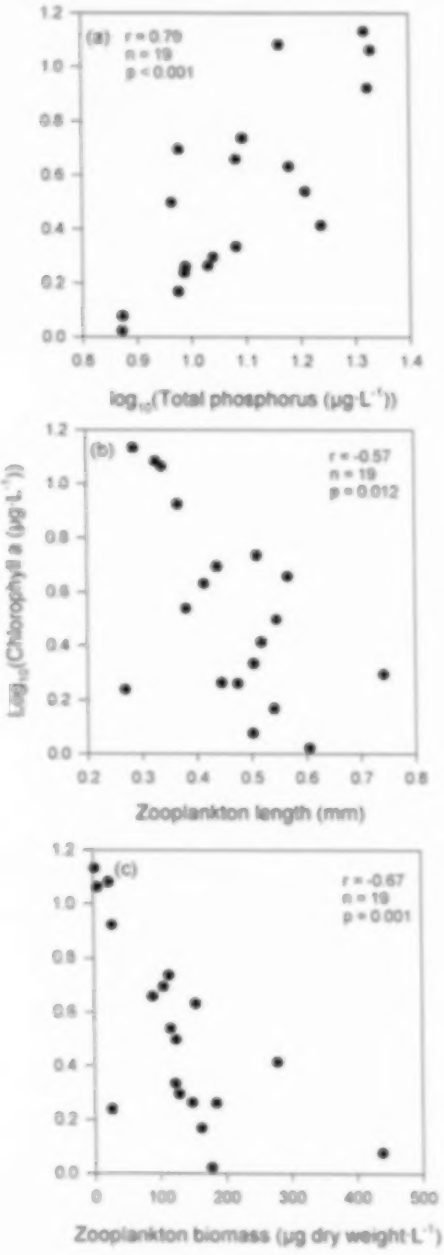


(r = –0.47, n = 19, p = 0.041) but did not appear related to lake surface area or in-lake biomass (r = –0.16, p = 0.52, and r = –0.13, p = 0.6, respectively).

The variables of interest in this study were significantly correlated with one another, as assessed by Pearson's correlation coefficient. As expected, there was a positive correlation between TP and Chl a (r = 0.79, p < 0.001; Fig. 4). Chl a was negatively correlated with ZB and ZL (r = –0.67, p = 0.001, and r = –0.57, p = 0.012, respectively; Fig. 4). TP was negatively correlated with both ZB and ZL (r = –0.529, p = 0.021, and r = –0.517, p = 0.023, respectively). The residuals of the TP–Chl a relationship showed a negative trend related to ZL (r = –0.25, p = 0.296) and ZB (r = –0.43, p = 0.068), though these correlations were not significant (Fig. 5). Additionally, ZL and ZB were positively correlated with one another (r = 0.486, p = 0.035). Although TP, ZB, and ZL co-vary, less than 30% of the variation in any given variable is explained by any other predictor.

Based on the hierarchical Bayesian model, correcting for measurement error, we found that TP and ZB were predictors of Chl a in our data set (Fig. 6). Bayesian credible intervals (95%) were calculated for each coefficient and those for TP (β_1) and ZB (β_2) did not overlap with zero ($0.63 \leq \beta_1 \leq 2.31$ and $-2.34 \leq \beta_2 \leq -0.08$). However, credible intervals for the ZL coefficient overlapped substantially with zero in both the full model (TP, ZB, and ZL) and a reduced model (TP and ZL only). This suggests that ZL had little or no effect on Chl a in our particular study. Analyses including surrogates or indices of zooplankton community size structure (mean length of cladocerans only, mean biomass per individual, percent of total biomass that is *Daphnia* spp.) were similarly not discernable from zero. The hierarchical Bayesian modeling approach returned qualitatively similar results to those produced by a traditional multiple linear regression analysis of the same data, but uncorrected for measurement error (Appendix B, Table B1). Therefore, the presence of an effect of ZB and the weak effect of ZL are not explainable by measurement error but instead seem to reflect ecological patterns in the data.

Fig. 4. Correlation between chlorophyll a and (a) total phosphorus (TP), (b) zooplankton length (ZL), and (c) zooplankton biomass (ZB) for northern Wisconsin lakes in late May and early June 2006. r, Pearson's correlation coefficient; n, sample size; p, p value (α = 0.05).



We compared the full model (TP, ZB, and ZL) with a reduced model (TP and ZB only) using the deviance information criterion (DIC) (Spiegelhalter et al. 2002) and the

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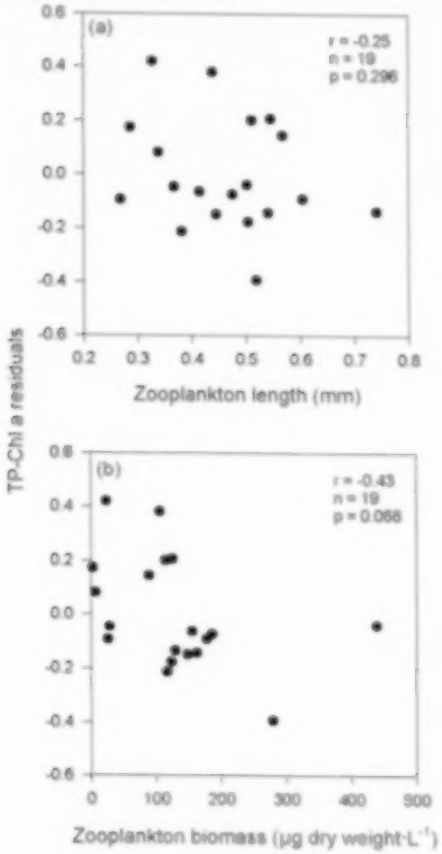

Fig. 5. Correlation between the residuals of the total phosphorus (TP) – Chl *a* relationship and (*a*) zooplankton length (ZL) and (*b*) zooplankton biomass (ZB). *r*, Pearson's correlation coefficient; *n*, sample size; *p*, *p* value ($\alpha = 0.05$).

multilevel R^2 (Gelman and Hill 2007). The DIC is a multilevel Bayesian analogue of the Akaike information criterion (AIC) (Akaike 1973) and is a measure of the fit of the model to the data corrected for the complexity of the model. The DIC for the full model was –980.8, and for the reduced model presented in Fig. 5, it was –557.6. The lower DIC for the full model suggests that it provides a better fit to the data. The corresponding multilevel R^2 for the full and reduced models were 0.67 and 0.68, respectively. These statistics suggest that although the full model fits the data better, inclusion of ZL does not improve the explanatory power of the model. There is as yet no consensus about the best measures of fit for complex hierarchical models (Clark 2007), and current practice is to calculate several different measures (Gelman and Hill 2007), as done here.

We compared the regression coefficients of models based on a single tow, thus uncorrected for measurement error, with those based on all tows combined and corrected for measurement error (Fig. 7). The estimated coefficients (given in eq. 1) and the uncertainties around these estimates did not differ largely between tows, and neither did the estimates based on individual tows differ from estimates based on the compilation of all five tows. As expected from measurement error theory, in the estimates derived from the individual tows (tow 1, 2, 3, 4, or 5), we observed a slight attenuating effect of measurement error on the estimated ZB coefficient (β_2) and a corresponding slight inflation of the estimated TP coefficient (β_1) compared with the estimates based on combining the data from all five tows and correcting for measurement error (All). However, these observed measurement error effects were small relative to the uncertainties about the estimated parameters (represented by the error bars in Fig. 7) resulting from the variability among lakes.

Discussion

Two results from our study are notable: (*i*) in contrast to several earlier studies, we observed an effect of crustacean ZB, but not ZL, on Chl *a*, and (*ii*) this finding does not appear to be related to the higher precision with which crustacean ZB was measured in our study, but rather to ecological processes or variability.

Several studies have found that accounting for interlake differences in ZL improves estimates of Chl *a* (Pace 1984; Evans et al. 1996; Mazumder and Havens 1998). Cladoceran zooplankton, in particular *Daphnia* spp., are notably effective grazers in freshwater pelagic systems (Peters and Downing 1984; Carpenter et al. 1996; Steiner 2001), and low levels of Chl *a* are often associated with the presence of large cladocerans (Shapiro and Wright 1984; Carpenter et al. 2001). We observed a large range in mean cladoceran length per lake (0.24–1.40 mm) that was comparable with the range displayed by studies in which ZL was a significant predictor using conventional regression (Indian River lakes (0.5–1.7 mm); Pace 1984). Although we observed a large range in zooplankton size among lakes, the effect of ZL in our data set was weak.

We found that indices related to zooplankton size (mean length of cladocerans only, mean biomass per individual, percentage of total biomass that is *Daphnia* spp.) also did not help explain the residual variation in Chl *a*. Because we focused on crustacean zooplankton and excluded rotifers and ciliates, the observed range of our zooplankton size indices may have been limited. We focused on crustacean zooplankton because these organisms make up the bulk of the zooplankton biomass in these lakes (Carpenter and Kitchell 1993) and seem to have the greatest effect on variability in Chl *a* within and among lakes (Carpenter et al. 1996). Pace (1984) measured both macro- and micro-zooplankton and derived an index of zooplankton community size based on the slope of the log–log relationships between zooplankton abundance and weight. The slopes of these lines were highly influenced by the inclusion of microzooplankton. The exclusion of microzooplankton from our study could potentially explain the lack of an observed effect using indices of zooplankton size.

Alternatively, Cyr (1998) showed that cladoceran- and copepod-dominated communities can maintain a similar effective grazing rate in low productivity systems. Many of our systems had reasonably low productivity, so it is possible that zooplankton community size structure did not influence total grazing pressure. This idea is further supported by

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Fig. 6. Distribution of estimated coefficients for the hierarchical Bayesian model. The solid line represents a fit to the full model (including total phosphorus (TP), zooplankton biomass (ZB), and zooplankton length (ZL)); the dotted line represents a reduced model (including only TP and ZB).

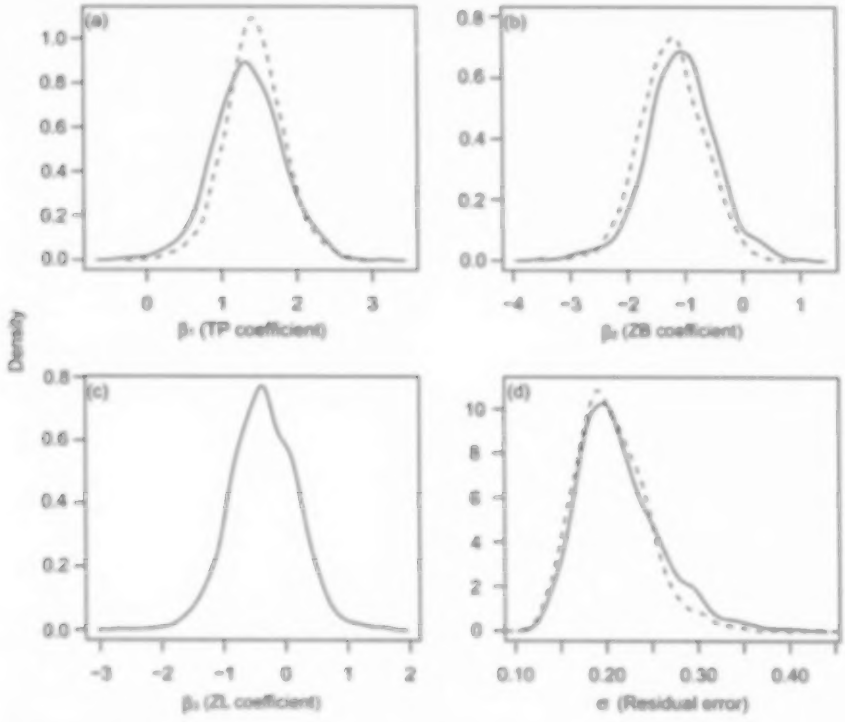
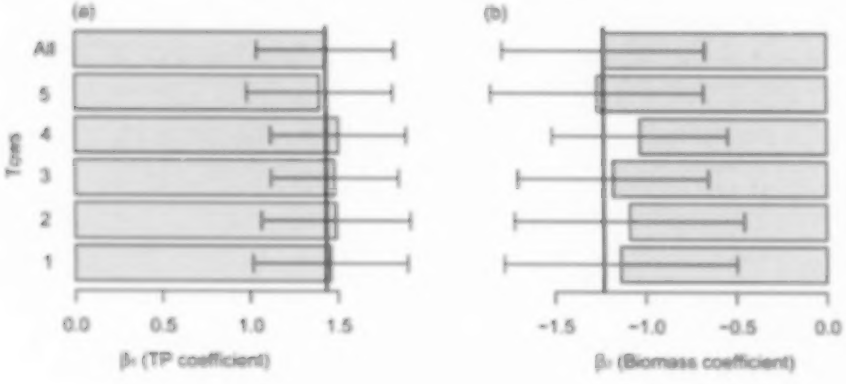


Fig. 7. Estimates of (*a*) total phosphorus (TP) and (*b*) zooplankton biomass (ZB) coefficient based on the reduced model (TP and ZB predictors only) and data from each individual tow (1, 2, 3, 4, 5) (uncorrected for measurement error) compared with using all tows combined (All) (corrected for measurement error). The solid vertical line indicates the parameter estimate that has been corrected for measurement error based on the mean TP or ZB from the five replicate tows in each lake. Deviation of individual tows above (TP) and below (ZB) this line suggests inflation of the coefficients for the more precisely measured variable (TP) and attenuation of the coefficients for the less precisely measured variable (ZB). The error bars represent variation among lakes (one standard error).



a cross-lake study in which zooplankton community grazing rates were affected by zooplankton biomass, but not zooplankton size distribution (Cyr and Pace 1992).

In contrast to several earlier studies (Pace 1984; Jeppesen et al. 2000), we found that crustacean ZB rather than ZL was a modest predictor of Chl *a* concentration in the lakes we sampled. Our study therefore suggests that in some contexts the zooplankton community can influence phytoplankton biomass independently of community size structure. Although ZB measurements are inherently impre-

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cise compared with measurements of ZL, our analyses do not suggest that our observation of a crustacean ZB effect was due to accounting for relative differences in measurement error. Regression coefficient estimates for crustacean ZB based on a single tow were only slightly attenuated compared with estimates based on the compilation of all five tows. Therefore, ZB was included as a predictor independent of the number of replicate tows included in the analysis.

The strength of the observed crustacean ZB relationship may vary over time because of the cyclic dynamics of the grazer–phytoplankton interaction (Carpenter and Kitchell 1987). We sampled single dates in early summer, whereas some other studies have used seasonal means to infer relationships. If dynamics of ZB and Chl *a* vary reciprocally over the summer, averaging over many dates may remove or obscure the dynamic relationship of ZB and Chl *a*. In such a case, short-term bottom-up and top-down dynamics that affect the biomass of the two communities may not be captured. This may not be the case for the ZL relationship if lakes with high ZL tend to have lower Chl *a* throughout the study period. Further research is needed to address the effects of averaging over time intervals of different lengths. Although time scale may affect the slope of the ZB–Chl *a* relationship (Carpenter and Kitchell 1987), our findings indicate that measurement error does not cause large bias in estimates of the slope from field data.

Our results do not support the hypothesis that measurement error weakens observed relationships of ZB to Chl *a*. Moreover, to better understand the drivers of phytoplankton biomass, it may be more important to sample a larger range of lakes over different time periods than to process replicate samples to reduce measurement error. Another compatible approach would be to collect a number of replicate samples per lake, but to pool these samples during the counting and measuring process. Such an approach would inherently capture much of the within-lake variation, yet eliminate the expenses associated with processing many individual replicate samples. Our study suggests that measurement error may not be an important bias, but we cannot infer that measurement error did not play a role in other studies that found zooplankton length and not biomass to be a significant predictor of Chl *a*. We could not test our hypothesis using data from previous studies because other studies collected fewer or no replicates and thus quantified measurement error with lower precision, preventing inclusion of these data in the hierarchical Bayesian model described here. Because we found that measurement error did not affect the results of this analysis, perhaps researchers should instead focus on other hypotheses that may affect zooplankton–Chl *a* relationships, such as diet breadth, numerical response, nutrient recycling, or other factors. Furthermore, our results suggest that studies of zooplankton–Chl *a* relationships should allocate limited research resources to sampling a wider range of drivers versus replication to correct for measurement error.

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Appendices A and B

Appendices A and B follow.

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Appendix A

Table A1. Length – dry weight regressions applied to samples in the study with a, b, c, and d constants in weight = a length^b or ln(weight) = c + d ln(length), where weight is measured in micrograms and length is measured in millimetres.

Species or genus	a	b	c	d	Source
Acanthocyclops spp.			2.0577	2.5530	Bottrell et al. 1976
Alona spp.	29.65	3.48			Dumont et al. 1975
Bosmina spp.	17.7369	2.2291			Culver et al. 1985
Ceriodaphnia			2.5623	3.3380	Bottrell et al. 1976
Chydorus spp.	14.0793	1.9796			Culver et al. 1985
Copepodites			2.0577	2.5530	Bottrell et al. 19766
Copepod nauplii	3.0893	1.7064			Culver et al. 1985
Daphnia pulicaria or pulex			1.9445	2.72	O'Brien and deNoyelles 1974
Daphnia retrocurva	3.7847	2.6807			Culver et al. 1985
Daphnia spp.			1.9445	2.72	O'Brien and deNoyelles 1974
Diacyclops thomasi	5.6713	1.9347			Culver et al. 198585
Diaphanosoma birgei			1.6242	3.0468	Bottrell et al. 1976
Diaptomus spp.	6.1927	1.9604			Culver et al. 1985
Epischura lacustris			1.467	2.4741	Lawrence et al. 1987
Eucyclops agilis			2.0577	2.5530	Bottrell et al. 1976
Holopedium gibberum[a]			–17.08	3.52	Yan and Mackie 1987
Leptodora kindtii	1.5605	1.873			Culver et al. 1985
Mesocyclops edax			1.3472	3.0087	Lawrence et al. 1987
Tropocyclops prasinus			2.0577	2.5530	Bottrell et al. 1976

[a]The length measurement for *Holopedium gibberum* is the distance between setae natatores and the terminal claw of the postabdomen (in μm). The equation to calculate total length was postabdominal claw length (μm) = 191.64 total length (mm) + 37.00 (Yan and Mackie 1987).

Appendix B

Table B1. Results of multiple linear regression analysis (not corrected for measurement error) predicting mean chlorophyll a concentrations from total phosphorus (TP), total volumetric zooplankton biomass (ZB), and mean individual zooplankton length (ZL).

	Coefficients	Standard error	t statistic	p
Intercept	–0.646	0.570	–1.135	0.274
TP	1.345	0.399	3.372	0.004
ZB	–1.084	0.548	–1.979	0.066
ZL	–0.334	0.474	–0.704	0.492

Note: n = 19, R^2 = 0.73, p < 0.001.

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A practical framework for selecting among single-species, community-, and ecosystem-based recovery plans

Mark S. Poos, Nicholas E. Mandrak, and Robert L. McLaughlin

Abstract: Science-based approaches for selecting among single-species, community-, and ecosystem-based recovery plans are needed to conserve imperilled species. Selection of recovery plans has often been based on past success rates with other taxa and systems or on economic cost, but less on the ecology of the system in question. We developed a framework for selecting a recovery plan based on the distributions and ecology of imperilled and nonimperilled species across available habitat types and applied it to fishes in the Sydenham River, Ontario, Canada. We first tested whether distributions of fishes were adequately predicted by habitat features hypothesized to limit the distributions of imperilled fishes versus a broader set of habitat features known to predict fish distributions. We then tested whether imperilled species occurred in similar or disparate habitat types. For the Sydenham River, an ecosystem-based recovery plan was deemed most appropriate because imperilled species occur in disparate habitat types. We lastly provide decision criteria to facilitate applications of our framework to the selection of recovery plans for other species and systems.

Résumé : Afin d'assurer la conservation des espèces en péril, il est nécessaire de posséder des méthodes basées sur la science pour choisir entre les plans de récupération centrés sur une seule espèce, sur une communauté ou sur un écosystème. Le choix d'un plan de récupération se base souvent plus sur les taux de succès obtenus avec d'autres taxons et dans d'autres systèmes ou sur le coût économique, mais moins sur l'écologie du système en question. Nous avons mis au point un cadre de sélection d'un plan de récupération basé sur la répartition et l'écologie des espèces menacées et non menacées dans les différents types d'habitats disponibles et nous l'avons utilisé dans le cas des poissons de la rivière Sydenham, Ontario, Canada. En premier lieu, nous avons vérifié si les répartitions des poissons pouvaient être adéquatement prédites d'après les caractéristiques de l'habitat que nous soupçonnions limiter les répartitions des poissons menacés, par comparaison à celles obtenues à partir d'un éventail connu et plus étendu de caractéristiques de l'habitat capable de prédire les répartitions de poissons. Nous avons ensuite vérifié si les espèces menacées se retrouvaient dans des types d'habitat similaires ou disparates. Dans la Sydenham, un plan de récupération à l'échelle de l'écosystème nous semble plus approprié car les espèces menacées vivent dans des types d'habitat disparates. Nous fournissons enfin des critères de choix pour faciliter l'utilisation de notre cadre dans la sélection des plans de récupération pour d'autres espèces et systèmes.

[Traduit par la Rédaction]

Introduction

Science-based approaches for selecting between recovery plans are needed to protect the growing number of imperilled species listed by conservation agencies. Federal agencies in Canada, the United States, Europe, Australia, and elsewhere are mandated by legislation to develop recovery plans for species listed as endangered or threatened (Tear et al. 1995). With the rate of species imperilment increasing (Miller et al. 1989; Ricciardi and Rasmussen 1999; Abbitt and Scott 2001), so is the need for management strategies necessary to facilitate recovery (Abell 2002; Lundquist et al. 2002). To date, decisions regarding the selection of recovery plan type have been based on comparisons of past success rates (Boersma et al. 2001; Clark et al. 2002) or economics (Tear et al. 1995), while decisions based on the given biotic community are rarely assessed and are sorely needed (Clark and Harvey 2002).

Recovery of imperilled species is typically implemented by conservation agencies using three recovery planning types: single-species, community-, and ecosystem-based plans. Single-species plans focus on the threats and recovery of an individual imperilled species (Franklin 1993; Caughley 1994). Although single-species plans are relatively successful, they are often expensive to implement (Campbell et al. 2002). Plans that focus on the recovery of more than one imperilled species or on the ecosystem in which those species live have been proposed as alternatives to single-species

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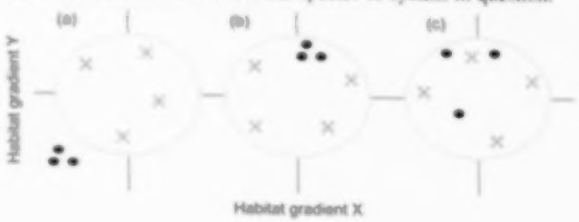
recovery plans (Grumbine 1997; Yaffee 1999; Clark and Harvey 2002). Community recovery plans facilitate the recovery of groups of imperilled species that share the same ecosystem or groups of taxonomically related species facing similar influences (Clark and Harvey 2002). Ecosystem-based recovery plans focus more broadly on imperilled and nonimperilled species and their habitats (Franklin 1993). Each type of plan has been used in the management of imperilled species recovery, with varying levels of success (Boersma et al. 2001; Pikitch et al. 2004). For any recovery planning type to be effective, it must consider the biology of the imperilled species, the factors affecting its distribution and abundance within a given community, and the biology of the other nonimperilled species (Boersma et al. 2001; Clark and Harvey 2002).

The development of a science-based framework for the selection of recovery plans intuitively begins with the description of the relationship between imperilled species and their habitat. Loss of critical habitat is the leading cause of species imperilment; consequently, greater than 99% of recovery plans target habitat protection as an objective for species recovery (Tear et al. 1995; Abbitt and Scott 2001; Dextrase and Mandrak 2006). However, as is often the case with imperilled species, biological information may be lacking and decisions for species recovery are needed regardless of data paucity (Foin et al. 1998). We use the identification of habitat-related factors (e.g., reduction, fragmentation, or modification) as a key starting point to discriminate between factors influencing imperilled species. We define imperilled species as those species designated as either threatened or endangered or those species given a conservation designation as suggested by the Committee for the Status of Endangered Wildlife in Canada. Although there are notable exceptions in factors that affect imperilled species (e.g., invasive species, disease, and over harvesting), our framework begins with the assumption that habitat-related factors are important first considerations when developing a recovery plan and that other factors can be included as they become known (see Dextrase and Mandrak 2006). Alternatively if other factors are known to influence the species or system in question, they should be included in the scenarios that we describe.

Three scenarios regarding the distribution of imperilled and nonimperilled species across available habitat types can be initially used to select a suitable type of recovery plan (Fig. 1). The first scenario considers an assemblage or community where imperilled species occur in a subset of habitats that are unique relative to those used by nonimperilled species (Fig. 1*a*). This scenario might be expected in systems where imperilled species are indicative of changes to specific habitats (Landres et al. 1988; Lawler et al. 2002). For example, declines in imperilled or sensitive species are often an indicator of broader habitat changes (e.g., habitat fragmentation in birds; pH in invertebrates and fish), where the larger suite of more common species has yet to be impacted (Jackson and Harvey 1993; Herkert 1994). For this scenario, single-species and community recovery plans could be implemented easily because mitigating the same set of habitat variables could be proposed to facilitate the recovery of all of the imperilled species (Fig. 1*a*). However, if the habitat variables are altered, the risk of nonimperilled species being negatively affected remains.

Fig. 1. Three scenarios (*a*–*c*) representing a framework of the habitat associations of imperilled and nonimperilled species. Solid circles represent individual imperilled species, Xs represent nonimperilled species, and the larger, open circles represent variation in all other taxa in the assemblage. Habitat gradients (axes) represent the factors of concern for the species or system in question.



The second scenario considers an assemblage or community where imperilled species occur together in a subset of the habitats used by nonimperilled species (Fig. 1*b*). This scenario might be expected where a system-wide habitat change negatively affects the entire suite of species or affects a subset of sensitive species. For example, changes in water temperatures through deforestation or loss of riparian zones are thought to affect both imperilled and nonimperilled species (France 1997; Casselman 2002). In this situation, single-species and community recovery plans would be well suited to facilitate the recovery of imperilled species because habitats could be modified to benefit all imperilled species (or a subset of sensitive species); accordingly, alterations to assist the recovery of imperilled species would have smaller effects on nonimperilled species because of the overlap in habitat preferences (Fig. 1).

The third scenario considers a system where different imperilled species occur in subsets of habitats and their geographic distributions overlap with nonimperilled species (Fig. 1*c*). This scenario is likely to occur where many environmental factors contribute to species declines. Under this scenario, single-species and community recovery plans may impede recovery of other imperilled species, as well as negatively affect nonimperilled species. For such a scenario, ecosystem-based recovery plans would be better suited than single-species or community recovery plans because improvements at a watershed–ecosystem level would presumably cause improvements to the myriad of species found in that watershed–ecosystem. Single-species or community recovery plans would only be feasible if the distributions of imperilled species were restricted to different geographical units (e.g., subwatersheds), where each could be managed separately for the threats occurring there. These three scenarios represent key examples from a continuum of scenarios, and specific scenarios and variables (e.g., other threats) can always be implemented as they occur.

We used four steps to explicitly apply our framework to imperilled fish species in the Sydenham River, Ontario, Canada (Fig. 1). We applied the framework to the Sydenham River watershed because this 2725 km^2 watershed is being used as a model system to develop scientific tools needed to support recovery planning for imperilled fish species, as required by the Canadian Species at Risk Act (Dextrase et al. 2003; Staton et al. 2003). The Sydenham River is species-rich for a north temperate system, with over 80 na-

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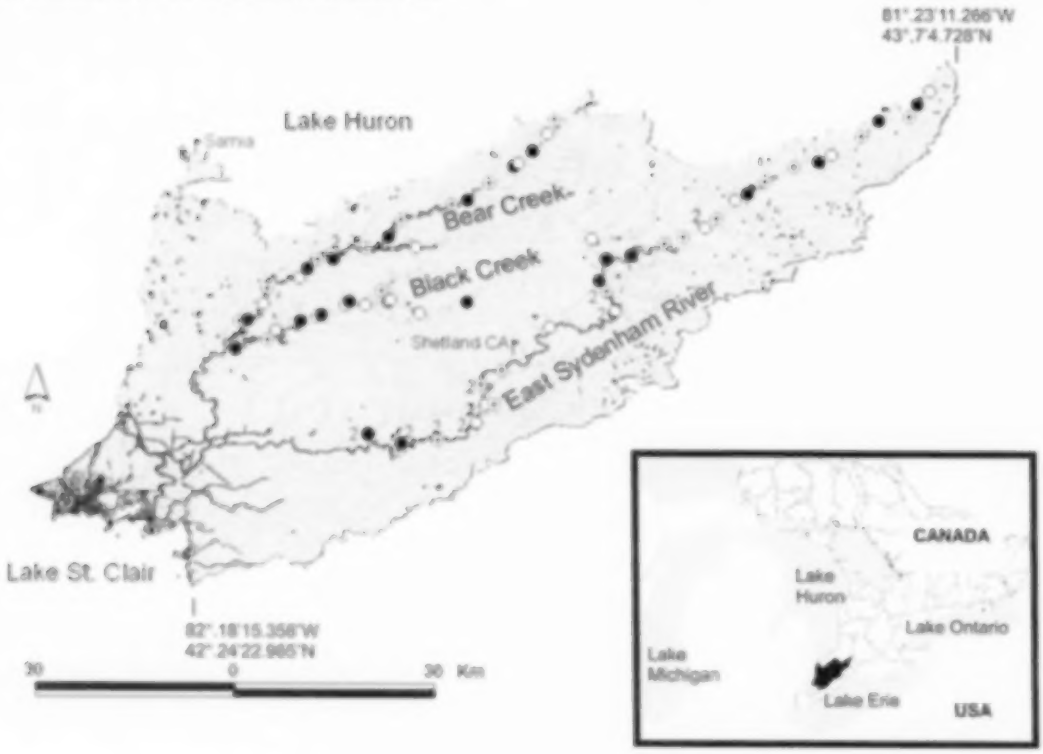
Fig. 2. Sites sampled (n = 75) in the Sydenham River, Ontario, in 2002 and 2003. Open circles represent locations sampled in 2002, solid circles are locations sampled in 2003, and open circles with black centers are locations sampled in 2002 and 2003. Numbers next to circles represent multiple sites in close proximity to one another. The inset shows the location of the Sydenham River relative to the Laurentian Great Lakes. Shetland CA is the Shetland Conservation Area.



tive and introduced fish species. First, we identified habitat features hypothesized in the literature to influence imperilled fish species in the Sydenham River. These species include the endangered northern madtom (*Noturus stigmosus*), the threatened eastern sand darter (*Ammocrypta pellucida*) and spotted gar (*Lepisosteus oculatus*), and species of special concern: greenside darter (*Etheostoma blennioides*), pugnose minnow (*Opsopoeodus emiliae*), bigmouth buffalo (*Ictiobus cyprinellus*), and blackstripe topminnow (*Fundulus notatus*). These species are all currently encompassed within one recovery plan and are therefore considered collectively (Dextrase et al. 2003; Staton et al. 2003). Second, we tested whether those habitat features identified in the literature were good predictors of the types of habitats where the imperilled species were found. We conducted this test because recovery plans typically rely on species accounts from the literature without explicitly examining the adequacy and efficacy of the habitat features hypothesized to be influencing the distributions of imperilled species in the system they are managing. Third, we examined the habitat associations of imperilled and nonimperilled species to test among the three scenarios depicted in Fig. 1. Finally, we examined whether imperilled species were restricted to different subwatersheds that could be managed separately, as posed under scenario 3 (Fig. 1c).

Materials and methods

Site selection

Seventy-five sites were sampled across the Sydenham River watershed. Fifty sites were sampled in 2002, 25 of these were resampled in 2003 along with 25 new sites (Fig. 2). Sixty-two sites, at least 2 km apart, were selected to achieve uniform coverage across the watershed. Non-wadeable sections of the river near Shetland Conservation Area and in the lower portions of the watershed were not sampled. We defined each site as either a pool–riffle sequence or a stream reach approximately 60 m in length. Because much of the watershed has been impacted by agricultural drainage (Dextrase et al. 2003), many stream reaches have become channelized, lacking pool–riffle sequences. When a sample site was located within a channelized reach, sites were defined as a 60 m stream reach, following Bohlin et al.'s (1989) recommendation to use sites of roughly equal length.

Fish sampling

Fishes were collected with an electrofisher, seine nets, gill net and overnight sets of Windermere traps. Each site was sampled using single-pass electrofishing with a backpack Smith-Root model 12 electrofisher and using a seine net.

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The type of seine net (bag or straight) used and the deployment of the remaining sampling methods were dependent on the depth of each site. A bag seine (8.2 m × 2 m × 2 m, 7.5 mm mesh), gill nets (5 cm stretched mesh), and Windermere traps (1 m diameter) were used at sites when depths were over 1 m. A straight seine (9.8 m × 2 m, 7.5 mm mesh) was used when depths were 0.5–1 m. A small straight seine (4.3 m × 0.6 m, 7.5 mm mesh) was used when depths were <0.5 m. Captured fishes were identified to species, counted, and either returned to the river (as the case for all imperilled species) or preserved as voucher specimens and sent to the Royal Ontario Museum (Toronto, Ontario).

Each site was systematically sampled by the electrofisher (pulsed DC current at 200–225 V, 60 Hz, pulse length = 3 ms) in an upstream direction at a rate of 5 electrofishing seconds per square metre (OMNR 2007). Total sampling effort depended on the area of the site; however, a minimum of 2000 s (mean 4257 ± 130 s) was shocked at each site, exceeding the recommendation of at least 1500 s proposed by Yoder and Smith (1999). The entire area of the site was sampled again by hauling a seine net in the downstream direction. The number of seine hauls varied from five to eight depending on the number of obstructions at the site. In total, the combination of electrofishing and seining captured over 95% of the imperilled species at sites where they were known to occur (Poos et al. 2007). Gill nets (5 cm stretched mesh) were used as block nets during sampling, unless a natural obstruction (e.g., shallow water) was present. Although absences were assumed as true absences because of the relatively large amount of sampling compared with others (e.g., Yoder and Smith 1999, OMNR 2007), we validated this assumption by modeling confidence intervals around each subsection and found all imperilled species had asymptotic relationships with a probability of detection >0.95 at the level we sampled (Poos et al. 2007).

Habitat measurement

Forty-two habitat variables were measured at each site. These represented habitat variables considered to specifically influence the distribution of imperilled fish species in the Sydenham River (Dextrase et al. 2003), as well as habitat variables considered to influence the distribution of stream fishes in general (Richter et al. 1997; Flather et al. 1998; OMNR 2007). Habitat variables were categorized as geomorphological, substrate, and chemical. Geomorphological variables measured attributes of the channel and stream hydrology and riparian buffers (OMNR 2007). Substrate variables measured the physical stream bottom where fishes were sampled (OMNR 2007), while chemical variables measured water quality at the sample sites (Table 1).

Geomorphological and substrate variables were measured using the Ontario Stream Assessment Protocol (OMNR 2007). This protocol was selected because it has proven useful for testing hypotheses regarding how fish communities respond to habitat change and for developing conservation strategies (Stanfield and Jones 1998). In-stream measurements were recorded at six equally spaced points along 10 equally spaced transects, totaling 60 in-stream measurements per site. In-stream measurements included hydraulic head (HH) (±1 mm) as an index of velocity, water depth (±1 mm, AvgDepth), average bank undercut (±1 mm), percentage of sample points with nonfilamentous algae (NFL), and percentages of sand (median particle size 0.1 mm; labeled as PrSand), cobble (median particle size 20 mm; labeled as PrCobble), and clay (median particle size 0.01 mm; labeled as PrClay). Riparian measurements included percent riparian buffer (PrVegSq), amount of aquatic grasses (PrGrass), average bank particle size (±1 mm), and average bank angle.

Water quality variables were measured using a HydroLab DataSonde 4a multiprobed sensor. The sensor measured specific conductivity (±0.001 $mS \cdot cm^{-1}$), turbidity (±50 nephelometric turbidity units, NTU), pH (±0.2), dissolved oxygen (±0.2 $mg \cdot L^{-1}$), and nitrate concentration (±2 $mg \cdot (l^{-N})^{-1}$). These measurements were made approximately 20 m above the upstream end of the sample site continuously for the duration of sampling. Measurements were averaged (± standard error) over the sampling duration for each site sampled.

Data analysis

Redundancy analysis was used instead of canonical correspondence analysis because a linear response model was considered appropriate given that the extent of species turnover (beta diversity) along the longest gradient of a detrended correspondence analysis (DCA) was less than 3 (Leps and Smilauer 2004). Further, the imperilled species captured in this study were all found in greater than 10% of the sites sampled (using all sampling methods) and as such did not suffer from zero-inflated bias as one would assume with most imperilled species (Martin et al. 2005). Species scores were standardized by dividing by their standard deviation so that species with large variance did not unduly influence the analysis (ter Braak and Smilauer 2002). Scaling was focused on interspecies correlations to interpret spatial relationships among species (ter Braak and Smilauer 2002).

The 42 habitat variables were reduced to a more interpretable number of variables in two steps. First, eight variables that were only suitable for measurement at a small number of samples sites (<0.1% of transect points) were removed. For example, the presence of watercress (*Rorippa nasturtium-aquaticum*), a groundwater indicator in temperate climates, revealed that the plant was found at only 0.02% of the transect points. Second, four derived variables were created by averaging multiple repeated measurements and using the average, instead of the individual measurements. These derived variables were average bank angle, average percent riparian buffer, average undercut, and average bank particle size. For example, the four riparian measurements of the slope of each bank were summarized as average bank angle. In total, 20 variables were included in the overall redundancy model (Table 1). A reduced redundancy model including only those habitat variables identified in the literature as influencing imperilled species found on the Sydenham River was also developed (Table 1). Statistical significance of individual axes was tested using Monte Carlo permutation tests (Leps and Smilauer 2004). After the first axis was tested, it was used as a co-variable in the test of the second axis, and so on, until all axes were tested for significance in explanatory power (Leps and Smilauer 2004). Only axes significant at $p < 0.05$ were included in subsequent sections.

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Table 1. Variables from the literature identified as influencing imperilled fish species in the Sydenham River, Ontario (reduced model) and stream fish assemblages in general (overall model).

Predicted variable (Measured variable)	Eastern sand darter[a]	Greenside darter[b]	Blackstripe topminnow[c]	Bigmouth buffalo[d]	Spotted sucker[e]
Reduced model					
Depth (AvgDepth)	0 (0)	− (−)	0 (+)	+ (+)	+ (+)
Flow (HH)	0 (+)	+ (+)	0 (−)	− (−)	− (−)
Nonfilamentous algae(NFL)	0 (+)	+ (+)	0 (−)	0 (−)	0 (−)
Riparian buffer (PrVegSq)	+ (+)	+ (+)	0 (−)	− (−)	0 (−)
Aquatic grasses (PrGrass)	0 (−)	0 (−)	+ (+)	0 (+)	+ (+)
Sand substrate (PrSand)	+ (0)	0 (+)	0 (−)	0 (−)	0 (−)
Cobble substrate(PrCobble)	0 (+)	+ (+)	0 (−)	0 (−)	0 (−)
Turbidity (AvgTur)	− (−)	− (−)	+ (+)	+ (+)	− (+)
Nitrate ($AvgNO_3$)	− (−)	0 (−)	0 (+)	0 (+)	0 (+)
Dissolved oxygen (AvgDO)	+ (+)	+ (+)	0 (−)	0 (−)	0 (−)
Overall model					
Clay substrate (PrClay)	0 (−)	0 (0)	0 (−)	0 (−)	0 (+)
pH (pH)	0 (−)	0 (0)	0 (−)	0 (−)	0 (+)
Average undercut	NA	NA	NA	NA	NA
Bank particle	NA	NA	NA	NA	NA
Average bank angle	NA	NA	NA	NA	NA
Average substrate size	NA	NA	NA	NA	NA
Gravel substrate	NA	NA	NA	NA	NA
Pebble substrate	NA	NA	NA	NA	NA
Boulder substrate	NA	NA	NA	NA	NA
Conductivity	NA	NA	NA	NA	NA

Note: Predicted and observed signs of the associations between presence of an imperilled species and the habitat variable are shown (+, a positive correlation; −, a negative correlation; 0, no correlation). Observed associations appear in parentheses. NA, not applicable.

[a] Williams et al. 1989; Daniels 1993; Holm and Mandrak 1996; Facey 1998.

[b] Scott and Crossman 1973; Grossman and Freeman 1987; Greenberg 1991.

[c] McKee and Parker 1982; McAllister 1987; Dextrase et al. 2003.

[d] Scott and Crossman 1973; Stewart et al. 1985; Goodchild 1990.

[e] Trautman 1957; Scott and Crossman 1973; Page and Burr 1991.

The usefulness of habitat variables identified from the literature as influencing the imperilled species found on the Sydenham River was assessed by comparing the reduced redundancy model with the overall redundancy model. The comparison was made by using the variables isolated in the reduced model as co-variables and testing whether addition of the remaining variables in the overall model significantly increased the proportion of variance explained. Statistical significance of the improvement was tested using an unrestricted Monte Carlo permutation test ($p < 0.01$). The permutation test determined whether the variance in species' occurrences explained by the additional habitat variables was greater than that expected by chance (Leps and Smilauer 2004).

Evaluation of the scenarios

Biplots of the first two habitat axes from the redundancy analysis were used to generate figures comparable to Fig. 1, visualize the habitat associations of imperilled and nonimperilled fishes, and distinguish among the scenarios. Locations where imperilled species were detected were plotted on maps using the geographic information system ArcView 9.1 (ESRI, Redlands, California) to determine whether the imperilled species were aggregations occurring in certain subwatersheds.

Results

We collected 67 fish species from the Sydenham River, including five imperilled species. In total 43 928 (2002 = 20 685; 2003 = 23 244) fishes were captured, with the imperilled species eastern sand darter, greenside darter, blackstripe topminnow, bigmouth buffalo and spotted sucker (*Minytrema melanops*) accounting for 0.19%, 9.20%, 1.10%, 0.07%, and 0.04% of the total abundance, respectively. We did not capture three imperilled fishes, the pugnose minnow, spotted gar, and northern madtom. Two of these species (northern madtom and spotted gar) are thought to be extirpated from this watershed, while the pugnose minnow was captured in nonwadeable sections not sampled in this study (N.E. Mandrak, unpublished data).

Habitat associations of imperilled and nonimperilled species were most consistent with scenario 3 (Fig. 1*c*). The imperilled species were found in contrasting habitat types interspersed throughout the range of habitats used by fishes without conservation designations. Moreover, the eastern

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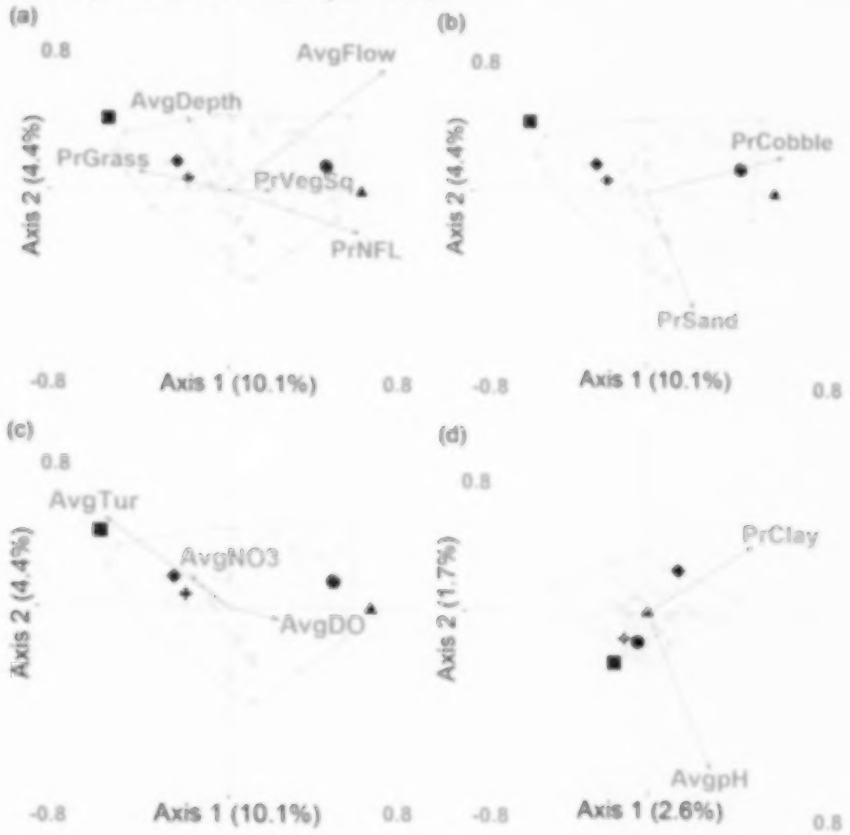
Fig. 3. Biplots from a redundancy analysis displaying the habitat associations of imperilled fishes (symbols) in the Sydenham River, Ontario, in 2002 and 2003 in relation to nonimperilled fishes (Xs) using habitat factors identified in literature as influencing imperilled fishes (reduced model) and subdivided as (*a*) geomorphological, (*b*) substrate, and (*c*) water quality variables and (*d*) additional variables thought to influence stream fish in general, but were shown to be good predictors of imperilled species (overall model). Percent variance explained by each axis is provided in parentheses. Species are shown by the following: circle, eastern sand darter; triangle, greenside darter; square, blackstripe topminnow; star, bigmouth buffalo; diamond, spotted sucker.



sand darter and greenside darter were found in habitats characterized by high hydraulic head (HH), and hence flow, large amounts of nonfilamentous algae (NFL), high proportions of cobble substrate (PrCobble), high amounts of riparian buffers (PrVegSq), and high concentrations of dissolved oxygen (AvgDO). Conversely, the blackstripe topminnow, bigmouth buffalo and spotted sucker were found in habitats characterized by deep water (AvgDepth), high turbidity (AvgTur), and high nitrate concentration ($AvgNO_3$).

The habitat variables hypothesized as important in literature accounts of the imperilled species were found to be good predictors of the occurrence of imperilled species in the Sydenham watershed. The redundancy analyses demonstrated that the model based on a broad suite of habitat variables known to influence stream fishes in general did not provide a significantly better fit then a model based solely on those habitat variables believed to influence imperilled species (14.5% for the reduced redundancy model versus 17.3% for the overall redundancy model, $p > 0.05$). In addition, there was a significant relationship between the habitat variables believed to influence imperilled species and the occurrence of the fishes in general (axis 1: eigenvalue = 0.1; $F = 5.3$; $p = 0.001$; axis 2: eignenvalue = 0.04; $F = 2.6$, $p = 0.002$).

Our consideration of variables not identified for imperilled species in the literature identified two additional variables that were helpful in predicting the occurrence of imperilled species in the Sydenham River. The variables were percent clay substrate (PrClay) and pH (Fig. 3*d*). Adding those variables to the reduced redundancy model explained an additional 2.6% of the variance in the occurrence of fish species (eigenvalue = 0.017, $F = 2.07$, $p = 0.003$; eigenvalue = 0.014, $F = 1.75$, $p = 0.01$, respectively). Only axis 1 was significant (axis 1: eigenvalue = 0.019; $F = 1.9$; $p = 0.01$; axis 2: eigenvalue = 0.012; $F = 0.87$, $p = 0.32$). The occurrence of the eastern sand darter, blackstripe topminnow, and bigmouth buffalo tended to be in habitats characterized by low amounts of clay and low pH. Spotted sucker also tended to be in habitats characterized by high amounts of clay and high pH. The occurrence of the greenside darter was unrelated to the amount of clay and water pH (Table 1; Fig. 3).

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Discussion

As rates of species imperilment and habitat degradation increase, meeting the challenges of species recovery has become increasingly difficult (Foin et al. 1998; Simberloff 1998). One strategy to circumvent this downward trend has been to reduce the emphasis on species-specific recovery plans (which require more effort per species) and use ecosystem-based recovery plans, where several imperilled species can be managed across entire systems, such as watersheds (Grumbine 1994; Brunner and Clark 1997). Although ecosystem-based recovery plans may be scientifically appropriate, the willingness of managers to adopt these strategies may be difficult given the past success of single-species recovery approaches, which have been four times more likely to exhibit recovering status trends for imperilled species (Boersma et al. 2001; Clark and Harvey 2002). Of the number of limitations addressed for shortcomings of ecosystem-based recovery plans, the lack of integration of ecologically relevant information into recovery plans are almost exclusively linked with lack of improved status (Tear et al. 1995; Clark et al. 2002). This may be more related to discrepancies between the type of recovery plan chosen and the specific conservation tasks identified for recovery in the biological system or species in question rather than a specific type of recovery plan (Lundquist et al. 2002). For example, the lumping of species into community plans is often not based on ecologically defensible criteria (e.g., similarity of threats) or represents inconsistent associations with preferred habitat ranges (Clark and Harvey 2002; Rahn et al. 2006), which is counterintuitive to the goals of that recovery plan. This study introduces a framework to improve the implementation of selecting an appropriate recovery plan type by providing a basis for incorporating species-specific associations directly into decision criteria (Fig. 4). The goal of using decision criteria is to incorporate an understanding of species–habitat relationships for the selection and implementation of an appropriate type of recovery plan.

Classification and validation of habitat variables thought to be critical for the conservation of imperilled species is fundamental to the presented framework because habitat characteristics often become key components of species recovery plans (Tear et al. 1995), under the assumption that they are important, but often without appropriate validation (Gerber and Hatch 2002). Our application quantitatively demonstrated that habitat variables identified in the literature as influencing the distributions of imperilled fish species found in the Sydenham River were good predictors of the habitats where those species were found; however, this scenario may not always occur. Validation of habitat variables can be specifically useful for isolating uncertain predictors or for identifying new habitat predictors not previously recognized in the literature. We consider the classification and validation of habitat characteristics as preliminary steps in the decision framework (Fig. 4). These steps are necessary for the success of the framework because plans that incorporate species-specific biology are typically more successful than those that do not (Boersma et al. 2001; Lawler et al. 2002; Lundquist et al. 2002). Further, identification of new, important habitat predictors of imperilled species (through validation procedures) may lead to the development of better recovery plans by improving our knowledge of habitat associations for imperilled and nonimperilled species. The discovery of previously unimportant habitat predictors will increase knowledge of the biology of species inhabiting the management area, thereby increasing rigor to the assessment of scenarios 1–3.

The framework and decision criteria presented here represent spatially explicit criteria from which to base management decisions for communities with imperilled species. By considering the effect of species threats with geographic location, there is a high likelihood that the success rate of community- and ecosystem-based plans can be improved. For example, we found that imperilled species in the Sydenham River were found in different habitat types and were not restricted to different subwatersheds, which suggests considerable challenges to recovery planning using community recovery plans. Such situations can be expected to pose constraints whereby actions taken to assist the recovery on one imperilled species will potentially hamper the recovery of another. In addition, strict calculation of threat similarity (Clark and Harvey 2002) may not encompass the geographic extent from which those threats should be mitigated. For example, the Sydenham River recovery strategy has focused on reducing turbidity to mitigate threats to critical habitat (Dextrase et al. 2003). Reducing turbidity throughout the watershed is predicted to benefit the eastern sand darter and greenside darter, because both species occur at sites characterized by low turbidity. However, reducing turbidity could negatively affect the blackstripe topminnow, bigmouth buffalo, and spotted sucker because these species occur at sites characterized by high turbidity. Single-species or community recovery plans could be effective if the eastern sand darter and greenside darter were primarily found in the less turbid eastern subwatershed and the blackstripe topminnow, bigmouth buffalo, and spotted sucker were primarily found in the turbid northern subwatershed. Such a scenario would require partitioning of recovery actions for each watershed so that each subwatershed could be managed differently. However, this is not the case in the Sydenham River, thereby, supporting selection of an ecosystem-based recovery plan and not a community plan (Fig. 4). Recovery actions focusing on single factor remediation, such as the reduction of turbidity, need to be tempered with the trade-offs in species-specific responses, specifically that any shift may be at the detriment of at least some imperilled species. Although this type of situation may ultimately doom ecosystem-based approaches to lower success rates (by reducing the viability of some imperilled species), presumably there would be a net benefit to the other imperilled and nonimperilled species by improving habitat quantity and quality through an ecosystem approach.

A stronger, science-based approach using decisions tailored to the organisms and system of concern can help with the selection of a recovery plan type. With the rate of species imperilment increasing (Miller et al. 1989; Ricciardi and Rasmussen 1999; Abbitt and Scott 2001), so is the need for recovery plans defining management strategies necessary to facilitate recovery (Abell 2002; Lundquist et al. 2002). To date, recommendations regarding the choice of recovery plan have been based on either the past success rates with other taxa and systems (Boersma et al. 2001; Clark et al. 2002) or economic cost of the different recovery plans

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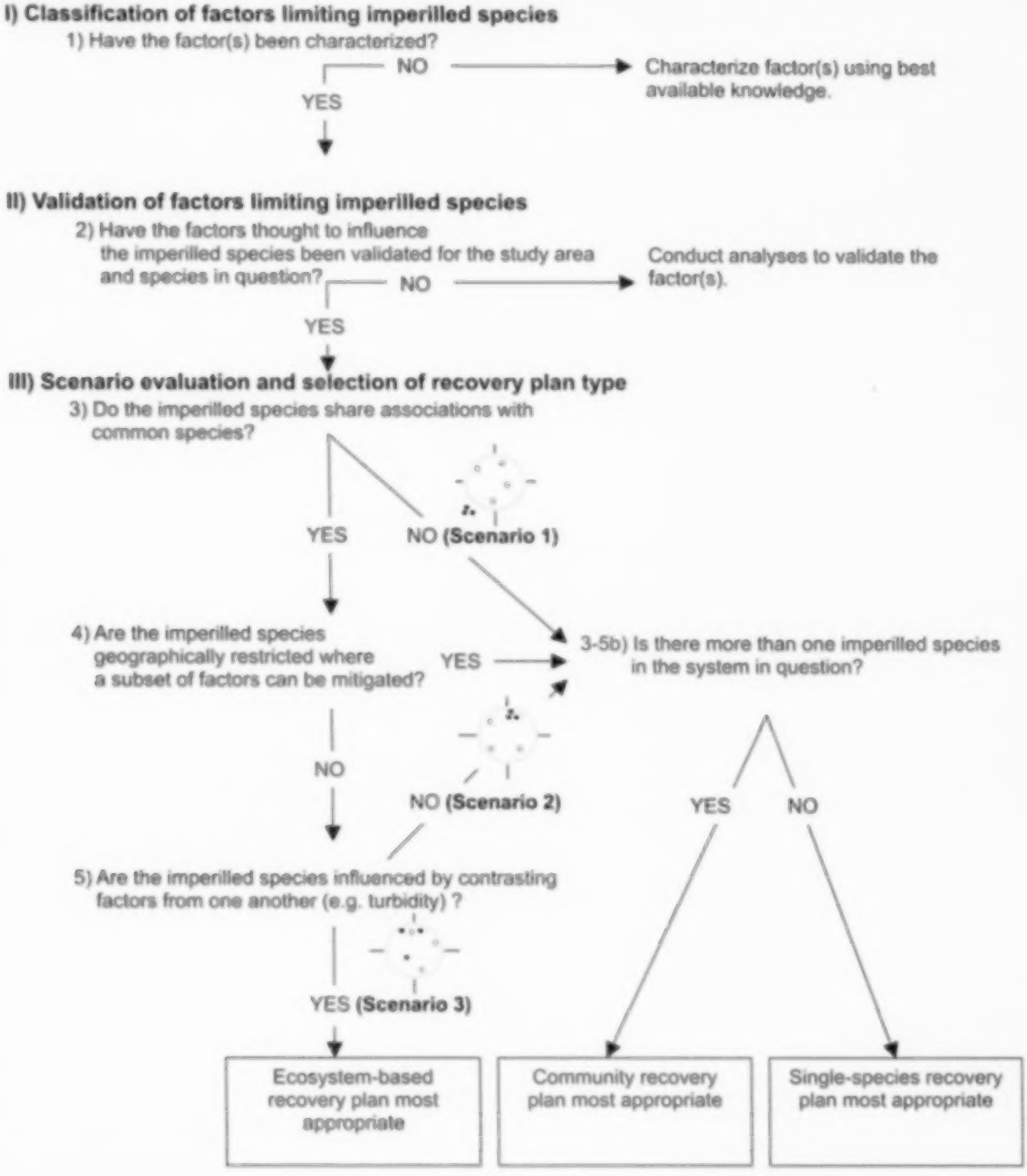
Fig. 4. Decision criteria for determining the appropriate recovery planning type for imperilled species based on species–habitat associations identified in Fig. 1.


I) Classification of factors limiting imperilled species
1) Have the factor(s) been characterized?
NO
Characterize factor(s) using best available knowledge.
YES
II) Validation of factors limiting imperilled species
2) Have the factors thought to influence the imperilled species been validated for the study area and species in question?
NO
Conduct analyses to validate the factor(s).
YES
III) Scenario evaluation and selection of recovery plan type
3) Do the imperilled species share associations with common species?
YES
NO (Scenario 1)
4) Are the imperilled species geographically restricted where a subset of factors can be mitigated?
YES
3-5b) Is there more than one imperilled species in the system in question?
NO
NO (Scenario 2)
YES
NO
5) Are the imperilled species influenced by contrasting factors from one another (e.g. turbidity) ?
YES (Scenario 3)
Ecosystem-based recovery plan most appropriate
Community recovery plan most appropriate
Single-species recovery plan most appropriate

(Tear et al. 1995) and not necessarily on the ecology of the system in question. While past success and economics are important considerations for choosing a type of recovery plan, they do not explicitly recognize that the success rate and cost of each type of recovery plan may vary according to the ecological situation in which the recovery plan is applied. For example, the higher success rates observed for single-species recovery plans relative to community plans found by Boersma et al. (2001) may be a consequence of the former being implemented in simple systems and of the latter implemented in more complex, challenging systems. Alternatively, the cost effectiveness of community recovery plans should be improved when the recovery plan incorporates knowledge of local conditions, and this should also im-

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prove the rate of recovery (Grumbine 1994). In addition, both single-species and community approaches may be more cost effective in situations where factors contribute similarly to communities with imperilled species, such as an increase in water temperature through the loss of riparian buffers, and where recovery can be achieved in a relatively short time frame (Lawler et al. 2002).

Of course, the framework developed has limitations and understanding these can help identify where they can be applied most successfully and how it might be improved. First, implementing the framework requires quantifying the species–habitat associations, but it does not demonstrate cause and effect relationships. Therefore, habitat modification as a recovery action, such as reducing turbidity, may not necessarily lead to corresponding changes in the abundances of imperilled species due to the complex ways in which species interact with the environment and the effects of other factors not measured, such as connectedness among suitable habitats. However, cause and effect could be tested subsequently either in formal field experiments or through adaptive management. Second, the variance explained from multivariate analyses (e.g., redundancy analysis, canonical correspondence analysis) will tend to be lower than their univariate counterparts (e.g., logistic regression, least squares regression, analysis of variance) and should not be taken as a surrogate for poor fit of the model per se (Legendre and Legendre 1998). In this case, the low variance on the multivariate axes represent the overall ability of the environmental correlates in describing the relatively large species assemblages (n = 67) across the relatively large number of sites sampled (n = 75) and as such is not surprisingly low. Although we should always strive for rigorous species assessments, in many cases extensive sampling of the kind preformed here may not be feasible. As is often the case with endangered species, biological information may be sparse for rare species and decisions for species recovery are needed regardless (Foin et al. 1998). In general, the models represent the integration of the best available knowledge across the study system and species in question. As the decision criteria presented here (Fig. 4) does not incorporate explicitly statistical methods, care should be used to ensure appropriate models are applied. Finally, we did not consider other imperilled taxonomic groups, such as mussels (Metcalfe-Smith et al. 2003) or benthic macroinvertebrates or the imperilled fishes not captured in our sampling. The addition of other imperilled species may provide more insight as to which habitat characteristics would be best mitigated in the situation where several imperilled species need to be alleviated with contrasting habitat types. Indeed, the majority of the declines attributed to imperilled mussels are thought to be due to turbidity (Dextrase et al. 2003). The inclusion of other imperilled species would, therefore, improve the implementation of an ecosystem-based recovery strategy, and such data should be included when implementing the most appropriate type of recovery plan. However, in our situation, adding the imperilled species not sampled would not have altered our results or the choice of which recovery plan type would be most appropriate. The reason for this is that as imperilled species are added to the multivariate data, the more likely that those species will be found with some aspect of habitat that is contrasting with another imperilled species without geographic isolation (scenario 3).

This framework has a number of strengths that may allow it be transferred to other species and systems. First, the framework for the selection of recovery plan type can be standardized and is straightforward and transparent. Second, the framework is rigorous by using strong science to validate proposed habitat predictors and to test among competing scenarios and is comprehensive by explicitly considering imperilled and nonimperilled species. Third, the framework presents opportunities to learn, for example, through identifying new habitat predictors to include in the community model (e.g., pH or percent clay in our model). Fourth, the framework lends itself to an adaptive approach as biological information becomes more available, limiting factors become validated, and extinction risks become better quantified.

Finally, the framework is flexible in the use of most multivariate statistical methodologies, insofar that they are used appropriately and can distinguish between the scenarios we present. These advantages may lead to improved success with species recoveries by lessening the use of inappropriate recovery plans that are based solely on past success and tight fiscal resources (Grumbine 1994, 1997; Boersma et al. 2001). As the recovery of imperilled species will be hindered if the objectives of recovery plans cannot be met (Boersma et al. 2001; Lundquist et al. 2002) and as loss of imperilled species rises, it is becoming increasingly important that conservation actions, such as the selection of recovery plans, be transparent, sound, and scientifically defensible.

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Relating stream physical habitat condition and concordance of biotic productivity across multiple taxa

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Abstract: To explore the potential of assessments of stream geomorphic condition and habitat quality in evaluating lotic productivity, we investigated concordance of stream biotic productivity (aquatic macroinvertebrates, crayfish, fish, and belted kingfishers (*Ceryle alcyon*)) and their physical habitat correlates in 18 streams in the Champlain Valley, Vermont, USA. Pearson correlation analysis indicated significant concordance between macroinvertebrate density and fish biomass ($r = 0.76$), between the density of macroinvertebrates in the orders Ephemeroptera, Plecoptera, and Trichoptera and fish biomass ($r = 0.81$), and between fish biomass and kingfisher brood weight ($r = 0.54$). We used principal component analysis followed by linear regression to investigate relationships between physical habitat condition and biotic productivity and to identify key components of physical habitat condition assessments. Our analysis supported the combined use of geomorphic and habitat assessments as a comprehensive indicator of stream physical habitat condition. We found relationships between habitat assessment scores and productivity measures of all taxa except crayfish, suggesting similar responses to physical condition across trophic levels. Our results encourage the use of additional taxa, in addition to widely used macroinvertebrate metrics, as indicators of the composite effects of physical habitat impairment in stream ecosystems.

Résumé : Afin d'examiner le potentiel des caractéristiques géomorphiques et de la qualité d'habitat d'un cours d'eau pour en évaluer la productivité, nous déterminons la concordance entre la productivité biotique (macroinvertébrés aquatiques, écrevisses, poissons et martins-pêcheurs d'Amérique (*Ceryle alcyon*)) et les variables physiques associées de l'habitat dans 18 cours d'eau de la vallée du lac Champlain, Vermont, É.-U. Une analyse de corrélation de Pearson indique une concordance significative entre la densité des macroinvertébrés et la biomasse des poissons ($r = 0,76$), entre la densité des macroinvertébrés des ordres des Éphéméroptères, des Plécoptères et des Trichoptères et la biomasse des poissons ($r = 0,81$), ainsi qu'entre la biomasse des poissons et la masse des couvées de martins-pêcheurs ($r = 0,54$). Une analyse en composantes principales suivie d'une analyse de régression linéaire a servi à déterminer les relations entre la condition physique de l'habitat et la productivité biotique et à identifier les éléments essentiels pour l'évaluation de la condition physique de l'habitat. Notre analyse appuie l'utilisation d'une évaluation combinée de la géomorphologie et de l'habitat comme indicateur global de la condition physique d'un habitat d'eau courante. Nous trouvons des relations entre les mesures d'évaluation de l'habitat et l'importance de la productivité chez tous les taxons à l'exception des écrevisses, ce qui laisse croire que les réactions aux conditions physiques sont semblables à tous les niveaux trophiques. Nos résultats appuient l'utilisation de taxons additionnels, en plus des métriques de macroinvertébrés largement employées, comme indicateurs des effets globaux de la détérioration de l'habitat dans les écosystèmes d'eau courante.

[Traduit par la Rédaction]

Introduction

The link between aquatic biota and their physical environment is widely recognized (Petts et al. 1995; Barbour et al. 1999; Montgomery 1999). Both fish (Karr 1981; Deacon and Mize 1997; Sutherland et al. 2002) and macroinvertebrates (Kerans and Karr 1994; DeShon 1995; Yoder and Rankin 1995) are commonly used as indicators of stream ecological condition. Aquatic birds are also increasingly being used in assessing stream health (Bryce et al. 2002; Buckton and Ormerod 2002; Sullivan et al. 2007). Because of their apex trophic position and dependence on aquatic resources, belted kingfishers (*Ceryle alcyon*) are particularly susceptible to changes in stream condition (Sullivan et al. 2006*a*). Primarily feeding on fish of 11–13 cm in length and using crayfish as a supplemental food source, belted kingfishers (hereafter, "kingfisher") require in-stream habitat that supplies sufficient food resources both in and out of breeding season (Brooks and Davis 1987; Hamas 1994).

Whereas many studies commonly focus on one taxon,

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using multiple taxa may produce additional ecological understanding through examination of interactions of different assemblages with the physical environment (Jackson and Harvey 1993; Allen et al. 1999; Wright and Li 2002). As human activities continue to place ecological systems under stress, consideration of various assemblages will also provide information regarding the relative vulnerability of these taxa to habitat changes.

Biological productivity (measured as production per unit area; Walters et al. 1997) is a straightforward measure of relative ecosystem health that can be expected to reflect both the quality and availability of environmental resources (Lowe et al. 1986; Bjornn and Reiser 1991; Garman and Moring 1993), as well as imbalances in the trophic structure of aquatic communities (Kawasaki 1992; Nickelson et al. 1992; Welcomme 1995). Although food web theory predicts trophic level connectivity (Polis and Winemiller 1996; Pimm 2002), both natural and human disturbances alter stream ecosystems in ways that restructure these connections and may differentially affect individual trophic levels (Power et al. 1996; Townsend and Riley 1999; Lake 2000). Our goal was to investigate how composite geomorphic and habitat assessments may relate to similarities (or differences) in productivity across trophic levels. We recognize that some differences among stream ecosystems are not captured by these assessment measures, including differences in basic water chemistry and water quality among sites.

In this study, we investigated (*i*) concordance of biotic productivity of aquatic macroinvertebrates, crayfish, fish, and kingfishers (i.e., degree of agreement in patterns of productivity of each taxon) and (*ii*) how stream physical habitat (measured using a combination of geomorphic and habitat assessment protocols) related to the observed concordance. Our null hypothesis was that we would see no concordance in productivity across the four taxa.

Materials and methods

As part of a larger study of the links between geomorphic condition and kingfisher reproductive ecology, we measured breeding territory length and brood weights at a suite of stream reaches in Vermont's Champlain Basin (Sullivan et al. 2006*a*). During the reproductive season, both male and female kingfishers actively defend their breeding territories and have been shown to reverse direction at the end of their territory and return to the middle when followed (Davis 1982). Therefore, we were able to measure the breeding territory length of each pair by walking along the stream, flushing the resident kingfishers to fly ahead of the observer. We flagged the upstream and downstream boundaries of each territory. Because territories follow the stream's longitudinal course, we measured the territories in length (metres) instead of area. Lateral boundaries were determined by the tree line along the edge of the channel.

Study area

In 2002 and 2003, we conducted our investigation relating to concordance patterns of biotic productivity at 18 kingfisher breeding territories. These territories were designated as our study reaches and, from north to south, spanned a distance of approximately 140 km.

The study reaches represented a range of physical characteristics and conditions (Table 1), but were located in areas with similar landform and elevation. Reach length varied from 638 to 2908 m. All streams were second to fifth order, and most were in the transfer zone (Schumm 1977) of the watershed. The specific measures of stream geomorphology and habitat quality varied significantly from site to site (Table 1), providing a range of conditions suitable to test our hypothesis.

Vermont's Champlain Basin is dominated by forest (64%), followed by agriculture (16%), open water (10%), urban areas (6%), and wetlands (4%) (Lake Champlain Basin Program (LCPB) 2004). Because of this land-use pattern, physical disturbance and sedimentation comprise the primary human impacts on area streams. Discharge of pollutants in Vermont streams is also an issue in some locations, but none of the study reaches received significant point-source discharges or were listed as impaired by any specific pollutants (including pH) under Section 303d of the US Clean Water Act. Anthropogenic activities leading to removal of riparian vegetation, hydrologic modifications, floodplain encroachments, channel straightening, and streambank erosion are thus the principal causes of changes in stream condition at our study reaches and in most of Vermont (Vermont Department of Environmental Conservation (VTDEC) 2001).

Physical habitat condition: geomorphic and habitat assessments

At all reaches, we conducted field geomorphic and habitat condition assessments following the Vermont Department of Environmental Conservation's protocols (VTDEC 2003), and supplemented these with additional parameters (Sullivan et al. 2004).

The Vermont Rapid Geomorphic Assessment (RGA) is a field-based, stream assessment methodology with a geomorphic evaluation, a channel evolution model (Schumm et al. 1984), and a stream classification using the Rosgen (1994) and Montgomery and Buffington (1997) systems. We characterized each stream reach following the guidelines established by the Vermont RGA and Assessment Field Notes and assigned a score from 0 (worst condition) to 20 (optimal condition) for each of four geomorphic adjustment processes: channel degradation (incision), channel aggradation, overwidened channel, and change in planform (VTDEC 2003). The scores from these four categories were then aggregated to form the composite or overall RGA score. Additional geomorphic measures included bankfull width, maximum depth, flood prone width, mean depth, and meander pattern following Rosgen (1994) and VTDEC (2003) geomorphic assessment protocols.

Assessments of habitat quality were based on the Vermont Rapid Habitat Assessment (RHA) protocols (VTDEC 2003). The Vermont RHA is derived from the US Environmental Protection Agency's Rapid Bioassessment protocols (Plafkin et al. 1989; Barbour et al. 1999) and is principally aimed at capturing simplification of habitat diversity across the reach. Specifically, we evaluated epifaunal substrate and available in-stream cover (i.e., structures on the streambed that provide habitat and cover), degree of embeddedness (i.e., amount of surface area of cobbles, boulders, snags,

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Table 1. Stream and river reaches and their primary physical characteristics and lengths.

Stream or river reach	Stream order	Watershed zone	Sinuosity	Stream morphological type	Predominant bed substrate	Reach length (m)	PHC score
Black River	4	Response	High	Pool–riffle/dune–ripple	Sand	1297	146
Browns River A	4	Transfer	Moderate	Pool–riffle/dune–ripple	Sand	2908	90
Browns River B	4	Transfer/response	Moderate	Pool–riffle/dune–ripple	Sand	983	151
Fairfield River	2	Source/transfer	Low–moderate	Pool–riffle	Gravel	1980	115
LaPlatte River A	4	Transfer	Moderate	Pool–riffle	Gravel	1060	173
LaPlatte River B	4	Transfer	Moderate	Pool–riffle	Gravel	1920	121
LaPlatte River C	5	Transfer	Low–moderate	Pool–riffle	Sand–gravel	1349	139
LaPlatte River D	5	Transfer	Moderate	Pool–riffle	Gravel	923	172
LaPlatte River E	5	Transfer	High	Pool–riffle	Gravel	1323	157
LaPlatte River F	5	Transfer	Moderate	Pool–riffle	Sand–gravel	1589	126
Lewis Creek A	4	Transfer	Very high	Pool–riffle	Sand	1772	133
Lewis Creek B	4	Transfer	High	Pool–riffle/dune–ripple	Sand	1598	146
Lewis Creek C	4	Transfer	Low–moderate	Pool–riffle	Gravel	1654	123
Lewis Creek D	4	Transfer/response	Moderate	Pool–riffle/dune–ripple	Gravel–sand	1384	166
Mallets Creek	2	Transfer	High	Pool–riffle	Gravel–cobble	638	179
New Haven River	3	Transfer	Moderate	Pool–riffle	Gravel	1912	114
South Branch	2	Source	Low–moderate	Step–pool/pool–riffle	Bedrock–cobble	1306	129
Tyler Branch	3	Transfer	Moderate	Pool–riffle/plane bed	Bedrock–gravel–boulder	2252	107

Note: Stream order based on US Geological Surveys 1:24 000 topographic maps. Watershed zone based on Schumm (1977). Sinuosity is based on meander pattern where low is <1.2, moderate is 1.2–1.5, and high is >1.5. Reach lengths are based on kingfisher breeding territories. PHC, physical habitat condition.

and other stream-bottom structures covered with sand and silt), representation of a heterogeneous mixture of velocity and depth regimes (i.e., diversity of flow patterns), amount of sediment deposition, status of channel flow (i.e., degree to which the channel is filled with water), degree of channel alteration (e.g., berms, dredging, straightening, and streambank armoring), frequency of riffles, bank stability, vegetative protection (i.e., amount of vegetative protection on the streambank and in the near-stream portion of the riparian zone), and the width of the riparian vegetative zone. We evaluated these parameters through field assessments following the guidelines and indicators of the Vermont RHA (VTDEC 2003) for each category. From these assessments, we assigned each habitat parameter included in the RHA a value from 0 to 20. When aggregated, these individual scores yield an overall habitat evaluation ranging from 0 to 200. Higher assessment values indicate better habitat conditions.

To adjust for differences in maximum scores (i.e., habitat assessment, maximum score = 200 points; geomorphic assessment, maximum score = 80 points), we recalculated scores for each metric based on a 100-point maximum score and then summed the two, equally weighted assessment scores. For input into our statistical analyses, we combined the geomorphic and habitat assessment scores into a physical habitat condition (PHC) score.

Biological assessment metrics commonly include physical characteristics that contribute to multiple categories within the calculated index (Karr 1981; Lyons et al. 1995; Barbour et al. 1999), leading to a degree of redundancy among the variables. However, based on their widespread use in habitat and geomorphic metrics, we included all variables. Additionally, whereas one variable may target current condition, a related variable may target mechanism or function.

Biotic sampling

Our measures of biotic productivity consisted of macroinvertebrate assemblage density, density of EPT (Ephemeroptera, Plecoptera, and Trichoptera) orders, crayfish assemblage biomass and density, fish assemblage biomass and density, and kingfisher clutch size and brood weight at fledging.

Kingfishers

During the 2002 and 2003 reproductive seasons, we counted the number of eggs after incubation began. We then visited kingfisher nests every second or third day until the eggs hatched. After hatching, we weighed and banded each nestling with US Fish and Wildlife Service bands. Nestlings were subsequently weighed at six to seven days and every fourth day thereafter until fledging (Sullivan et al. 2006*a*).

Aquatic macroinvertebrates

We collected macroinvertebrates from the beginning of July to mid-August, sampling at six locations per reach. Our primary criterion was to proportionally represent the composition of flow habitats (e.g., riffles, runs, pools) of the reach at large (e.g., for a reach comprised of 50% run and 50% riffle, three sampling locations in run habitat and three in riffle habitat would be selected). To the degree possible, we distributed sampling locations at equidistant incre-

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ments along the reach length. Organisms were collected using a 600 cm^2 Surber sampler with a 500 μm mesh net by disturbing the substrate and scraping larger rocks for 90 s intervals. Samples were preserved in 70% ethanol. In the laboratory, the entire sample was sorted, counted, and identified to order using Merritt and Cummins (1996) as a guide.

Fish and crayfish

To reflect the flow composition of the overall reach, we selected three to five sampling locations representative of the flow habitats (VTDEC 2004; Sullivan et al. 2006*b*). We varied the number of sampling locations in proportion to the reach length so that 12%–18% of the wetted area of each reach was sampled, giving us a consistent and proportional sampling effort at each reach. Both preliminary work conducted in the region (pre-study) and other investigations (Patton et al. 2000; Magalhaes et al. 2002) indicated that we were approximating an appropriate effort required to produce a representative assemblage. Using dipnets and a 1.22 m × 12.19 m bag seine with 3.175 mm mesh weighted with lead lines to prevent fish and crayfish from escaping, we sampled fish and crayfish at each selected location, combining all samples from each reach to characterize the fish and crayfish assemblages. We counted all fish and crayfish, using these data to generate density estimates; and we weighed all crayfish, basing biomass estimates for crayfish on these data. For fish assemblage biomass estimates, we used an in-field fixed-count subsampling protocol (Platkin et al. 1989; Growns et al. 1997) modified for fish, randomly selecting a subsample of 150 individual fish from each collection location at each study reach. We weighed the 150 fish in each subsample to the nearest 0.1 g and based assemblage biomass estimates for fish on the 150-fish subsamples (Sullivan et al. 2006*b*).

Numerical and statistical approaches

For each reach, total macroinvertebrate assemblage density and EPT density were generated by calculating the mean number of individual insects from the six Surber sample collections, which yielded the number of insects per square metre × 10^{-3}. Density estimates for fish and crayfish were based on total number of individuals per total cubic metres sampled. Using calculations of mean individual weight from the fish subsamples and the total number of fish caught per cubic metre, we calculated the total reach biomass for fish.

We performed Pearson correlations on the productivity measures of the four assemblages to isolate concordant patterns and followed this with principal component analysis (PCA) on the correlations (see Allen et al. 1999). We also performed PCA on all individual geomorphic and habitat assessment categories that contributed to the PCH score (see *Table 2*, italicized variables). In both cases, we retained PCA axes with eigenvalues > 1 (Snedecor and Cochran 1967; Rencher 1995; Walters et al. 2003).

We then built models in stepwise linear regression using (*i*) the retained PCA axes of biological productivity as response variables, and (*ii*) PHC score, stream order, bankfull width, mean depth, and the retained PCA axes of physical characteristics as predictor variables. Because one of our primary goals was to understand the composite effects of combined habitat and geomorphic characteristics on biotic productivity, we input PHC score as an individual variable. Stream order, bankfull width, and mean depth were also kept as individual variables because we considered a priori that these variables might be of particular importance in affecting biotic productivity. Variable additions proceeded until the F statistic for the change at the step fell below the $p < 0.05$ significance threshold.

We considered using individual metrics of biotic productivity and physical habitat directly in multiple regression models. However, we selected PCA because it allowed us to analyze the effects of physical habitat on concordant patterns of biotic productivity versus on a taxon-by-taxon basis and because of its ability to reduce the number of physical habitat variables and identify the most important gradients among them (Rencher 1995).

We performed all statistical analyses using JMP® 5.0 Statistical Discovery Software (SAS Institute Inc., Cary, North Carolina). When necessary, transformations ($\log(x + 1)$, x^2, x^3, $x^{1/3}$) were used to normalize data and eliminate heteroscedasticity prior to analysis (Snedecor and Cochran 1967; *Zar* 1984). Potential outliers were identified and removed using Cook's D (Kleinbaum et al. 1998). We identified one outlier in fish biomass at Lewis Creek C.

Results

Correlation analysis

Analysis of concordance among productivity measures revealed a number of significant correlations (Table 3): density of the overall macroinvertebrate community was strongly correlated with fish community biomass ($r = 0.76$, $p = 0.0003$), as was the density of the EPT orders ($r = 0.81$, $p = 0.0001$); fish community biomass was positively correlated with kingfisher brood weight at fledgling ($r = 0.54$, $p = 0.030$) and weakly correlated with kingfisher clutch size ($r = 0.42$, $p = 0.093$). We also observed a weak relationship between macroinvertebrate density and kingfisher brood weight ($r = 0.41$, $p = 0.103$). We found no correlation between crayfish biomass or density and any other taxonomic productivity measure. Although we did not find complete concordance of biotic productivity among the four taxa, we did reject the null hypothesis of no concordance of biotic productivity.

Principal component analysis

Concordance of productivity

The PCA of the eight productivity measures identified three axes (Prod1, Prod2, and Prod3) with eigenvalues > 1 (Table 4). Prod1 explained about 50% of the variance and represented the dominant pattern of productivity among the taxa. Whereas fish biomass ($r^2 = 0.84$) and macroinvertebrate density ($r^2 = 0.76$) had the highest correlations with this axis, all but crayfish biomass ($r^2 = 0.05$) and crayfish density ($r^2 = 0.01$) had correlations greater than 0.50.

The second principal component (Prod2) captured about 24% of the taxonomic covariation that remained. Crayfish biomass ($r^2 = 0.84$) exerted the greatest influence on this axis (Table 4). Prod3 was most strongly associated with

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Table 2. Variables analyzed, their minimums, medians, and maximums in their original units, and their means and standard deviations (SD), after transformation where appropriate, for the study reaches.

	Original units				Values analyzed	
Explanatory and response variables	Minimum	Median	Maximum	Transformation	Mean	SD
Aggradation	*2.00*	*14.00*	*19.00*	*None*	*12.28*	*4.98*
Bank stability	*10.00*	*14.50*	*19.00*	*None*	*14.44*	*2.33*
Bankfull width (m)	2.50	17.65	51.00	$x^{1/3}$	17.30	6.07
BEKI: clutch size (no. of eggs·100 m^{-1})	0.47	0.40	0.78	none	0.47	0.17
BEKI: brood weight (g·100 m^{-1})	38.01	69.16	129.00	*None*	75.11	30.09
Channel alteration	*8.00*	*15.50*	*20.00*	*None*	*15.11*	*3.50*
Channel flow status	*10.00*	*15.00*	*17.00*	*None*	*14.72*	*1.90*
Crayfish biomass ($g·m^{-2}$)	0.00	0.01	9.11	log(x + 1)	0.20	0.56
Crayfish density (no.·m^{-2})	0.00	0.00	0.83	log(x + 1)	0.10	0.21
Embeddedness	*6.00*	*12.50*	*18.00*	*None*	*12.50*	*3.65*
Epifaunal substrate and cover	*5.00*	*14.50*	*18.00*	*None*	*14.00*	*3.77*
EPT density (no.·10^{-3} m^{-2})	*7.80*	*119.50*	*493.83*	log(x + 1)	*4.58*	*1.12*
Fish biomass ($g·m^{-2}$)	0.50	7.95	47.90	log(x + 1)	2.16	1.08
Fish density (no.·m^{-2})	0.34	1.50	3.15	log(x + 1)	5.74	5.77
Incision/degradation	*6.00*	*14.50*	*19.00*	*None*	*13.83*	*3.82*
Macroinvertebrate density (no.·10^{-3} m^{-2})	32.60	369.10	1184.80	None	443.94	304.50
Mean depth (m)	0.20	0.50	1.50	$x^{1/3}$	0.86	0.14
Morphological diversity	*12.00*	*16.00*	*20.00*	*None*	*16.33*	*2.72*
PHC	89.75	136.00	178.75	None	138.13	25.02
Planform change	*5.00*	*15.50*	*20.00*	*None*	*14.28*	*4.25*
Riparian vegetation width	*2.00*	*13.50*	*20.00*	*None*	*13.11*	*5.46*
Sediment deposition	*1.00*	*13.50*	*18.00*	*None*	*12.33*	*4.67*
Stream order	2.00	3.00	4.00	log(x + 1)	1.33	0.16
Stream type	4.00	7.00	8.00	x^3	358.00	109.59
Vegetative protection	*7.00*	*14.50*	*20.00*	*None*	*13.94*	*4.29*
Velocity/depth regime	*9.00*	*16.50*	*20.00*	*None*	*16.06*	*3.02*
Widening	*4.00*	*13.50*	*19.00*	*None*	*12.72*	*4.43*

Note: Italicized variables are those analyzed in the physical habitat PCA. Each of these variables constitutes a category in the cumulative PHC assessment.

kingfisher brood weight, but with an r^2 of only 0.23 (Table 4).

Influences of PHC categories

Scores from 14 categories combined to form the PHC score (Table 2, italicized variables). The PCA on these physical habitat categories yielded four principal components (PH1, PH2, PH3, PH4) with eigenvalues > 1 (Table 5).

PH1 had all positive loadings (Table 5). All but three of the categories (incision, velocity/depth, and planform) had correlations greater than 0.35. PH2, in contrast, was highly correlated with incision and had moderate correlations with embeddedness and velocity/depth regime. PH3 was defined by generally low correlations, with planform (r^2 = 0.46) and velocity/depth (r^2 = 0.42) wielding a markedly higher influence on this axis than any other categories (≤0.15).

Regression models

Our two-phase model-building effort produced two viable models (A and B) (Table 6). In the first phase, we sought to identify concordant biotic productivity responses to the overall PHC score as well as to select stream characteristics. The PHC score had a strong positive association with Prod1 (model A). However, none of the other environmental predictors (i.e., bankfull width, mean depth, stream order) was significant in the model. Neither the PHC nor any of the individual measures of physical habitat had a significant linear association with either Prod2 or Prod3.

The second phase was aimed at understanding which of the PHC variables (represented by physical habitat PCs) influenced biotic productivity. Of the physical habitat PCs, only PH1 contributed to a significant model (B; Table 6). In model B, 59% of the variance in Prod1 was explained by PH1. This model suggests that productivity of all taxa except crayfish was influenced by the PHC categories. Bank stability, channel aggradation, and the availability of epifaunal substrate and cover appear to be among the most important physical characteristics influencing biotic productivity of macroinvertebrates, fish, and kingfishers; productivity was least influenced by channel incision, channel planform adjustments, and velocity/depth regimes.

Discussion

Although habitat assessments have been well developed (Platts et al. 1983; Plafkin et al. 1989; Barbour et al. 1996) and are widely used (DNRE 1997; McMillan 1998; Raven et al. 1998) in assessing stream biotic condition, the influence of geomorphic condition in conjunction with habitat condition may be underappreciated. By combining coordinated habitat and geomorphic assessments into a more comprehensive physical habitat condition score, we were able to

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Table 3. Pearson product-moment correlations among productivity measures of the four taxa.

	Macro density (no.·100 cm^{-2})	EPT density (no.·100 cm^{-2})	Crayfish biomass (g·m^{-2})	Crayfish density (no.·m^{-2})	Fish biomass (g·m^{-2})	Fish density (no.·m^{-2})	BEKI clutch size (no. of eggs·100 m^{-2})	BEKI brood weight (g·100 m^{-2})
Macro density (no.·10^{-1} m^{-2})	1.00							
EPT density (no.·10^{-1} m^{-2})	0.82**	1.00						
Crayfish biomass (g·m^{-2})	0.22	-0.03	1.00					
Crayfish density (no.·m^{-2})	0.18	-0.01	0.75**	1.00				
Fish biomass (g·m^{-2})	0.76**	0.81**	0.19	0.09	1.00			
Fish density (no.·m^{-2})	0.29	0.31	0.04	0.07	0.77**	1.00		
BEKI clutch size (no. of eggs·100 m^{-2})	0.37	0.29	-0.06	-0.07	0.42*	0.37	1.00	
BEKI brood weight (g·100 m^{-2})	0.41*	0.28	0.14	-0.04	0.54**	0.40	0.97**	1.00

Note: *, significance at 0.10; **, significance at 0.05.

uniquely explore associations with concordant patterns of stream biotic productivity.

Concordance of biotic productivity

For the most part, our observations support expected trophic links. The positive correlation that we observed between macroinvertebrate density and measures of fish community productivity affirm the dependence of many fish species on macroinvertebrate abundance (Kilgour and Barton 1999; Huryn and Wallace 2000; Imre et al. 2004), particularly on the larger-bodied EPT taxa. Likewise, our results show that kingfisher productivity is tightly linked to fish productivity, as supported by Cornwell (1963), Davis (1980), and Prose (1985). Although kingfishers have been reported to feed directly on some aquatic insects (Salyer and Lagler 1946; Davis 1982), the weak association that we found between macroinvertebrate density and kingfisher brood weight likely suggests indirect linkages (through fish) between macroinvertebrate and kingfisher productivity.

Crayfish were found at only nine of the 18 reaches, with no distinct physical features clearly associating with crayfish presence. Their limited distribution may partly explain the lack of concordance observed between crayfish and other taxa, suggesting that crayfish are patchy components of stream food webs. In fact, this is the case with kingfishers in particular, which tend to feed on crayfish when fish are inaccessible because of water depth or turbidity (Davis 1980).

Regression models: A

Fish biomass and macroinvertebrate density were the most influential measures on Prod1, which was the salient axis in the PHC model (model A). PHC, the composite score of all habitat and geomorphic metrics, explained 67% of the variance observed in Prod1. The results from this model support the growing use of both habitat and geomorphic assessments in evaluating stream condition. Although we did not measure additional factors such as biotic interactions, energy exchanges, and water chemistry or quality, these may explain additional variance in our models.

Among in-stream biotic assemblages, changes in stream fish biomass are generally the last changes in a progression of impairments in community composition and structure (Schindler 1987; Kilgour and Barton 1999). Given the demonstrated trophic link between fish and macroinvertebrates, we can infer that impacts from physical condition first affect the macroinvertebrate community, followed by fish and then kingfishers.

Kingfisher clutch size and brood weight, both key measures of reproductive output, loaded onto and highly correlated with Prod1, although not as strongly as fish biomass and macroinvertebrate density. Although kingfishers likely cue into stream features that correlate with fish abundance and availability (Davis 1982, Sullivan et al. 2006*a*), unlike fish and macroinvertebrates, kingfishers can forage outside the stream channel and feed in floodplain waterbodies and other lateral habitats (Hamas 1994) when necessary. This plasticity in feeding behavior, as well as their high degree of mobility, likely explains why measures of kingfisher productivity were not as tightly tied to PHC scores as were macroinvertebrate density or fish biomass.

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Table 4. Productivity PCA: eigenvalues and the percent variance captured by the principal components (eigenvalues > 1), along with each principal component's loadings and the proportion of the variance (r^2) each variable shared with the PCA axes.

	Prod1		Prod2		Prod3	
PHC metrics	**Loading**	**r^2**	Loading	r^2	Loading	r^2
Macroinvertebrate density (no.·10^{-3} m^{-2})	**0.43**	**0.76**	0.10	0.02	0.17	0.03
EPT density (no.·10^{-3} m^{-2})	**0.41**	**0.69**	–0.13	0.03	0.24	0.06
Crayfish biomass (g·m^{-2})	**–0.11**	**0.05**	0.67	0.84	0.06	0.00
Crayfish density (no.·m^{-2})	**–0.06**	**0.01**	0.53	0.52	0.61	0.39
Fish biomass (g·m^{-2})	**0.46**	**0.84**	–0.17	0.05	0.17	0.03
Fish density (no.·m^{-2})	**0.36**	**0.51**	–0.14	0.04	0.34	0.12
BEKI: clutch size (no. of eggs·100 m^{-1})	**0.38**	**0.58**	0.34	0.22	–0.41	0.18
BEKI: brood weight (g·100 m^{-1})	**0.38**	**0.58**	0.29	0.16	–0.47	0.23
Eigenvalue		**4.03**		1.88		1.04
% variance		**50.37**		23.54		13.06

Note: Principal components in bold type indicate those used as successful response variables in the regression models.

Table 5. Physical habitat PCA: eigenvalues and the percent variance captured by the principal components (eigenvalues > 1), along with each principal component's loadings and the proportion of the variance (r^2) each variable shared with the PCA axes.

	PH1		PH2		PH3		PH4	
PHC metrics	**Loading**	**r^2**	Loading	r^2	Loading	r^2	Loading	r^2
Aggradation	**0.32**	**0.65**	–0.10	0.02	–0.28	0.15	–0.14	0.03
Incision	**0.09**	**0.05**	0.55	0.69	0.19	0.07	–0.10	0.01
Planform	**0.19**	**0.22**	–0.20	0.09	0.49	0.46	0.11	0.02
Widening	**0.30**	**0.57**	–0.26	0.15	0.22	0.09	0.10	0.01
Bank stability	**0.32**	**0.64**	–0.05	0.01	–0.06	0.01	0.08	0.01
Channel alteration	**0.30**	**0.55**	–0.24	0.13	–0.21	0.09	0.18	0.05
Channel flow	**0.27**	**0.44**	–0.30	0.20	0.20	0.07	0.19	0.05
Embeddedness	**0.24**	**0.36**	0.37	0.31	–0.23	0.10	–0.27	0.11
Epifaunal substrate	**0.32**	**0.65**	0.04	0.00	0.16	0.05	–0.23	0.07
Morphological diversity	**0.30**	**0.57**	0.09	0.02	0.21	0.09	–0.39	0.22
Riparian vegetation width	**0.26**	**0.42**	0.27	0.17	–0.29	0.16	0.39	0.21
Sediment deposition	**0.30**	**0.57**	–0.15	0.05	–0.23	0.10	–0.40	0.22
Vegetative protection	**0.26**	**0.43**	0.25	0.14	–0.14	0.03	0.52	0.37
Velocity/depth	**0.13**	**0.11**	0.35	0.28	0.47	0.42	0.13	0.02
Eigenvalue		**6.21**		2.25		1.88		1.38
% variance		**44.32**		16.06		13.43		9.83

Note: Principal components in bold type indicate those that were significant predictors in the regression models.

Table 6. Explanatory variables and their coefficients in the significant linear regression models.

Model	Variable	Coefficient	R^2	F statistic
(A) Prod1 (n = 17)	Intercept	–8.89		
(P < 0.0001)	PHC	0.06	0.67	28.31
(B) Prod1 (n = 17)	Intercept	–0.06		
(P = 0.0003)	PH1	0.60	0.59	22.49

Crayfish, too, have the physiological and behavioral capacity to move into and out of the stream channel. Their weak, negative association with Prod1 and, therefore, with PHC scores suggests that this mobility, combined with a generalist feeding strategy and resilience to environmental stressors (Crocker and Barr 1968; Momot et al. 1978), may make them less vulnerable to habitat impairment than other taxa.

Fish density was less influential on Prod1 and, therefore, not as sensitive to changes in habitat and geomorphic conditions, likely because density may remain fairly constant in spite of changes in community composition (e.g., habitat generalists supplanting sensitive species) in impaired reaches. Similar findings are reported by Sullivan et al. (2006*b*).

Regression models: B

The second phase of our analysis addressed the individual contribution of each geomorphic or habitat metric to the overall PHC score and, ultimately, to biotic productivity. Loadings of equal value for each category (e.g., PHC metric) in PH1 would suggest that all categories exerted an equal influence on this PC axis. Because PH1 explained 67% of

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the variance observed in Prod1 (model A), equal loadings would suggest that all categories were important in influencing biotic productivity. Our results show that PH1 was, indeed, influenced relatively equally by most of the metrics, with the exception of incision and velocity/depth pattern. Overall, this pattern supports the inclusion of the component habitat and geomorphic metrics in stream assessments.

Channel incision had a markedly lower correlation (r^2 = 0.09) with this PC axis than any other variable, indicating that more than any other factor, channel impairment in the form of channel degradation did not affect biotic productivity. Channel incision is characterized by a lowering of the channel bed elevation through scour and often manifests in the form of regulated flows, chute cutoffs, inactive floodplains, and abandoned terraces. In our study, among the four types of channel adjustment evaluated (i.e., incision/degradation, aggradation, widening, planform change), we saw the smallest range in degrees of incision, spanning only 11 points (all other categories had at least a 15-point spread). This, in combination with the fact that the majority of incised sites in our study area were historically channelized (VTDEC 2001) and are currently readjusting and no longer down-cutting, likely mask some of the negative effects of channel incision on aquatic biota that are well documented (James 1991; Toth et al. 1998; Sullivan et al. 2006*a*).

Both categories relating to riparian vegetation (riparian vegetation zone width (i.e., extent of riparian zone – buffer width) and vegetative protection (i.e., streamside vegetation – streambank coverage, native vs. non-native, number of canopy layers, disturbance)) contributed virtually equally to PH1. In spite of the important contribution of allochthonous inputs to stream productivity (Vannote et al. 1980; Mantel et al. 2004), the contribution of the these riparian vegetation PHC categories influenced biotic productivity to a similar extent as did most other categories. It is possible that a threshold level of allochthonous inputs exists, only below which losses in biotic productivity are readily detectable. All of our stream reaches were located in riparian corridors that were characterized by a roughly even mixture of mixed deciduous–coniferous forest and open land (e.g., wet meadow, pasture, fallow land, etc.). This lack of a strong gradient of riparian vegetation and cover could have resulted in allochthonous inputs at all our reaches above this threshold amount.

The two most influential PHC categories influencing biotic productivity were bed aggradation and bank stability. High width to depth ratios, partial riffles and runs, midchannel bars, and chute cut-offs and channel avulsions are common features of aggrading reaches. In our reaches with little evidence of aggradation, we also tended to find limited sediment depositions in pools, low levels of embeddedness (i.e., amount of fine sediment surrounding gravel, cobbles, and boulders), and normal channel flows with water reaching the base of both lower banks during the low-flow period (August–September). Our results are not only in line with numerous other studies that report retarded macroinvertebrate and fish communities in streams with high sediment levels (Jones et al. 1999; Sutherland et al. 2002; Mol and Ouboter 2004), but also take these findings one step further to demonstrate effects on semi-aquatic birds as well. Reaches with compromised bank stability act as a point source for sediment input and contribute to bed aggradation. In contrast, a reach with good bank stability is characterized by minimal or absent erosion of bank failure with less than < 5% of the bank affected (VTDEC 2003).

Overall, our current results support the crucial role that physical habitat condition plays in stream ecosystems (Ward and Stanford 1979; Calow and Petts 1994) and provide new evidence regarding its role in governing reach-level faunal productivity. All categories that formed part of our PHC score contributed to some extent in explaining biotic productivity, although some were more influential than others. Bed aggradation was of particular importance and of notable applied significance given the ubiquitous increase in stream sediment loads due to timber harvest, road construction, and other human activities.

Although geomorphic assessments are currently used to investigate and understand watershed condition, develop and implement watershed management plans, prioritize stream reaches for restoration, and design subsequent restoration efforts (Gellis et al. 2001; VTDEC 2001; King and Day 2002), our results suggest that they will be vastly more informative and ecologically relevant when used in conjunction with habitat assessments. Together, these assessments provide a useful and cost-effective evaluation of stream physical habitat that is biologically important.

Although there is an implicit assumption in many assessments that impacts on benthic macroinvertebrates can also predict those on other aquatic organisms (Kilgour and Barton 1999), our results only partially support this conclusion. Overall, we found that macroinvertebrates, fish, and kingfishers were linked trophically and shared similar associations with physical habitat condition. Crayfish, however, appeared to be independent of these connections. Our results suggest that the response of biotic productivity to physical habitat changes in streams is complex, with purely aquatic–obligate organisms correlating most strongly with physical habitat condition. Given our increasing recognition of stream–riparian connectivity, as well as recognition of the stream and its corridor as an integrated ecological unit (Nakano and Murakami 2001; Baxter et al. 2005; Sullivan et al. 2007), our results indicate that physical habitat assessments that include more riparian, floodplain, and other lateral habitat metrics may be effective at capturing stream ecosystem condition.

We also have provided evidence that multitaxonomic approaches may be critical in assessing the physical condition of lotic systems. In particular, higher-level trophic taxa such as fish and even piscivorous birds may complement existing macroinvertebrate metrics by better representing realized differences in grain size and spatial scale (Roth et al. 1996; Finlay et al. 2002; Wright and Li 2002) and the bottom-up effects of resource availability (Power 1992; Polis and Winemiller 1996). Community structural aspects and mechanistic links, in addition to complete food webs, must also be explored to fully evaluate how physical habitat condition affects stream biotic productivity.

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Reproduction areas of roach (*Rutilus rutilus*) in the northern Baltic Sea: potential effects of climate change

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Abstract: The roach (*Rutilus rutilus*) is a common freshwater fish species in the brackish coastal areas of the northern Baltic Sea. In this study, surveys of roach larvae were carried out at reed-belt shores encompassing the entire archipelago zone in the northwestern Gulf of Finland. A logistic regression model was constructed and then used to spatially predict and map potential roach reproduction areas using a geographic information system (GIS). The results indicate that low spring salinity (<4‰) is the most important factor determining the success of roach reproduction. Reed-belt shores in the inner archipelago with large freshwater inputs in the spring constitute the key reproduction areas. Spring runoff peaks caused by melting snow together with the effects of ice cover on the spreading of freshwater runoff enable roach to reproduce relatively far from river mouths. Nevertheless, 68% of the reed-belt shores in the study area are presently beyond the 4‰ salinity limit and thus unsuitable for reproduction. In the future, climate change is predicted to reduce the salinity of the Baltic Sea, but the potential climate change impacts on roach are partly contradictory. The most likely outcome, however, is a spatial increase in the extent of roach reproduction areas in the northern Baltic Sea.

Résumé : Le gardon (*Rutilus rutilus*) est une espèce de poisson d'eau douce commune dans les eaux saumâtres des régions côtières du nord de la Baltique. Nous avons, dans notre étude, procédé à des inventaires des larves de gardons dans les ceintures littorales de roseaux dans l'ensemble de la zone des archipels du nord-ouest du golfe de Finlande. Nous avons élaboré un modèle de régression logistique et l'avons utilisé pour prédire l'emplacement des zones potentielles de reproduction des gardons et les cartographier à l'aide du GPS. Les résultats indiquent qu'une faible salinité au printemps (<4 ‰) est le facteur déterminant le plus important du succès de la reproduction des gardons. Les zones littorales à ceinture de roseaux dans l'archipel intérieur qui reçoivent de grands apports d'eau douce au printemps constituent les zones principales de reproduction. Les pics d'écoulement printanier causés par la fonte de la neige combinés aux effets de la couverture de glace sur la répartition de cet écoulement d'eau douce permettent aux gardons de se reproduire à une distance relativement grande de l'embouchure des rivières. Néanmoins, 68 % des zones littorales à ceinture de roseaux dans la région d'étude se situent présentement au-delà de la limite de salinité de 4 ‰ et sont ainsi impropres à la reproduction. Dans l'avenir, on prédit que le changement climatique réduira la salinité de la Baltique, mais les impacts potentiels de ce changement climatique sur le gardon sont en partie contradictoires. L'issue la plus vraisemblable, cependant, est une extension spatiale des zones de reproduction des gardons dans le nord de la Baltique.

[Traduit par la Rédaction]

Introduction

Roach (*Rutilus rutilus*) are common and widely distributed freshwater fish in northern Eurasia. Although of freshwater origin, roach also thrive in the brackish coastal and estuarine waters of the Baltic, Caspian, Black, and Aral seas (Bănărescu and Coad 1991). Adult roach are found in brackish water up to salinities of 10‰–14‰ (Jäger et al. 1981; Bănărescu and Coad 1991). Like other cyprinids, the roach is not an economically valued target species for the coastal fishery, but it is commonly caught by recreational fishermen, the annual recreational fishing catch being around 800 tonnes (t) on the Finnish coast alone (Anonymous 2004). In some countries, the roach is more valued also in the commercial catches (Erm et al. 1992). The roach is currently among the most abundant species in gill net test-fishing catches in the coastal areas of the northern Baltic Sea (Lappalainen et al. 2001; Ådjers et al. 2006), and its ecological importance in aquatic ecosystems is presumed to be considerable (Winfield and Townsend 1991).

The quality and quantity of spawning, larval, and nursery areas are important for fish production, and the habitat requirements of fish are most strict during the early life stages (Urho 2002). For roach, an improvement in reproduction conditions is the likely reason for the recent increase in abundance of the species in the Gulf of Finland. Shallow,

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sheltered, and vegetated shores are important as spawning and larval areas for roach in lakes (Holčík and Hruška 1965; Lange and Dmitriyeva 1973; Mann 1996). In the Baltic Sea, roach spawn in early May after the ice breakup, and during this time, shores covered by reeds (*Phragmites australis*) form the main vegetated habitat available for roach in the sheltered littoral shores of the northern Baltic Sea. The perennial reed belts extend from the hyperlittoral zone to a depth of usually 1.5 m, and new growth rises annually (Roosaluste 2007). Winter ice and waves typically cut down and flatten emergent reeds in the outer parts of the belt, forming a sheltered spawning and larval habitat for roach. The abundance and range of reed belts has increased, and they are a dominant feature of sheltered shores along the northern Baltic Sea coast (Roosaluste 2007). Reed expansion has been accelerated by human-induced climate change, by the cessation of coastal meadow management, and especially in the outer areas, by general eutrophication of the Baltic Sea (Roosaluste 2007).

According to laboratory experiments, the early life stages of roach are sensitive to salinity. Salinity is especially critical during fertilization and embryonic development and also for the survival of newly hatched roach larvae (Jäger et al. 1981; Klinkhardt and Winkler 1989). According to Jäger et el. (1981) and Klinkhardt and Winkler (1989), roach eggs fertilized in water with a salinity of 3.5‰ or higher have been found to die before the embryos hatch. Even if salinity was initially low and then increased to 3.5‰ or higher after hatching, the mortality rate of the newly hatched larvae was observed to be almost 100%. At lower salinities (<3.5‰), embryo development and hatching were normal. For Caspian roach (*Rutilus rutilus caspicus*), optimal salinity during reproduction has been reported to be 2.5‰–5‰ (Oliphan 1941), and for Black Sea roach (*Rutilus rutilus heckeli*), 0‰–5‰ (Karpevich 1975). These subspecies have clearly adapted themselves to more saline environments and, thus, have been experimentally shown to manage to reproduce in salinities up to as high as 7.5‰–10‰ (Oliphan 1941; Karpevich 1975).

Experimental studies have shown that a constant 9 °C forms a critical temperature minimum for the development of roach embryos (Černý 1974; Gulidov and Popova 1979). Roach abundance at the end of the first summer has also been reported to show a positive correlation with increasing turbidity, and eutrophic conditions have been suggested to be beneficial to roach in the early life stages (Sandström and Karås 2002).

Global climate change is expected to cause extensive changes in the aquatic ecosystem and fisheries (e.g., Kennedy 1990; Reist et al. 2006; BACC Author Team 2008). Nonetheless, insufficient information is available on the potential direct and indirect effects of climate change on fish. Attention so far has focused mainly on the effects of climate warming and increasing water temperatures on fish communities and species distributions (e.g., Lehtonen 1996; Reist et al. 2006; Henderson 2007). In the Baltic Sea, however, climate change is expected to influence not only the temperature, but also the salinity conditions (Meier 2006; Meier et al. 2006; BACC Author Team 2008) because of increased rainfall and increased freshwater runoff in the coastal areas (Carter et al. 2005; Silander et al. 2006). In the brackish water of the Baltic Sea, several aquatic organisms are living close to their salinity tolerance limit. Therefore, it appears that future changes in salinity will also considerably affect fish and other aquatic organisms in the Baltic Sea area.

The extent of the reproduction habitats of freshwater fish species such as roach is poorly known in the northern Baltic Sea, and no systematic surveys of the reproduction areas of roach have been carried out in the coastal areas. To date, only a few studies have used statistical modelling combined with geographic information system (GIS) to map essential fish habitats (e.g., Eastwood et al. 2001; Vaz et al. 2005; Bergström et al. 2007). However, continuous high-resolution maps of fish reproduction areas could offer versatile tools for efficient management of the coastal zone, and spatial predictive modelling would offer a convenient way to extrapolate the data gained by limited field survey resources. Thus, in this study, we combined remote sensing, field surveys, statistical models, and GIS methods to (*i*) determine and explain the occurrence of roach larvae along environmental gradients and (*ii*) produce spatial prediction maps of the potential reproduction areas of roach in an archipelago area in the northwestern Gulf of Finland. Finally, we considered the possible mechanisms by which climate change may affect spring salinities and consequently the extent of roach reproduction areas in the northwestern Gulf of Finland.

Materials and methods

Study area

The study area was located in the Tammisaari archipelago in the northwestern part of the Gulf of Finland (59 N, 23 E) and stretched from the innermost archipelago (Pojo Bay) to the outermost mainland (Hanko Peninsula). It was divided into three subareas representing the three archipelago zones: (A) the innermost bay area, (B) the intermediate archipelago, and (C) the outer archipelago (Fig. 1). The total length of the shoreline was 270 km in the inner bay area, 520 km in the intermediate archipelago, and 610 km in outer archipelago. The archipelago in this area is very complex and extensive. Environmental factors such as salinity, temperature, and littoral vegetation differ both spatially and temporally in the various archipelago zones, and strong environmental gradients are formed from the inner bays to the outer archipelago zone. Shores covered with reeds constitute a considerable part of the shoreline, especially in the shallow nearshore waters in the inner bay area: in the study area, reed belts extended along 58% of the shoreline in the inner bay area, 32% in the intermediate archipelago, and 9% in the outer archipelago. The River Mustionjoki, with a mean runoff of 19 $m^3 \cdot s^{-1}$, is the only large river in the study area and runs into the northern end of the innermost bay area. Consequently, a strong salinity gradient defines the study area, with salinity increasing from almost 0‰ to 6‰ at the outermost sites. Winter ice usually covers the area from January to April.

Selection of the study sites

On the basis of prior knowledge of the importance of reed belts as a reproduction habitat for roach (Mills 1991), we concentrated the field surveys on reed belt covered shores. The locations of shores with reed belts in the study area

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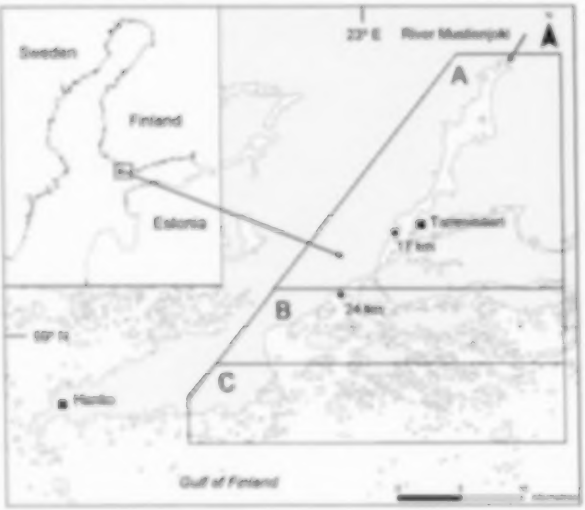
Fig. 1. Location of the study area and the three subareas on the southwestern coast of Finland: A, the innermost bay area; B, the intermediate archipelago; and C, the outer archipelago. Salinity sampling stations at 17 km and 24 km from the river mouth are marked with solid circles.



were visually determined using black and white aerial orthophotographs in digital format, provided by the National Land Survey of Finland. These aerial photographs were taken during the spring in 1997 and 2000 from a height of 6000 m and have a resolution of approximately 0.5 m. The locations of the reed belts were digitized into GIS format with ArcGIS (ArcMap 9.2; ESRI, Redlands, California). The study sites, comprising 100 m long stretches of reed-belt shores, were selected by stratified random sampling among the reed-covered shores in the three subareas.

The field survey took place between early May and mid-June during two consecutive years, 2004 and 2005. Altogether, 38 sites were selected in 2004, and 53 sites were selected in 2005, with 33 being the same in both years (Fig. 2). Additionally, 15 sampling sites in 2005 were located between the inner bay and the intermediate archipelago area to cover the entire coastal salinity gradient (1‰–6‰) and especially the part of the gradient that appeared to be critical to the reproduction of roach.

Sampling of roach larvae

Roach larvae were sampled by wading in the reed belt in the water depth range of 20–100 cm and seeking the larvae using a white plate and a white scoop (for more details, see Lappalainen et al. 2008). White plate and scoop methods have been developed to sample unevenly distributed larvae in dense vegetation such as reed belts (Hudd et al. 1983; Urho et al. 1990; Urho 1997). They are rapid and cost-efficient methods and can be used to determine the presence or absence of roach larvae at the study sites. Each 100 m long site was visited at least three times at 8- to 10-day intervals, with a sampling duration of 0.5 h per study site using two samplers. At each site and visit, 100 m of shore was checked by crisscross searching. The water temperature increased earlier in the inner areas, and therefore first visits to the inner subarea took place two weeks earlier than first visits to the outer subarea.

When larvae were present, larvae numbers varied between tens of individuals to schools of thousands of individuals. Roach eggs and newly hatched larvae (length ~7 mm) were found among the flattened reeds in the inner shallow part of the reed belt, whereas older larvae formed schools and swam in deeper water on the newly grown reed belt, reaching the outer edge of the belt. A few individuals were always collected with a scoop to verify species identification in the laboratory.

Background information and data analysis

The surface-water temperature and salinity were measured at each site and visit using a microprocessor-based conductivity and TDS meter (Hanna Instruments Limited, Bedfordshire, UK). Water turbidity at the outer edge of the reed belt was measured with Hach 2100 P meter (Hach Company, Loveland, Colorado). The mean width (from land to sea) of the reed belt at each site was measured in the field with a Garmin 76CS GPS (Garmin Limited, Olathe, Kansas). The water depth at the outer edge of the flattened reeds was measured, and the data were adjusted according to the current sea water level. The exposure of a site to wave action was estimated using an effective wave exposure index (fetch) (see Isæus 2004) with a 25 m resolution.

Logistic regression models were constructed to examine the association between the occurrence of larvae and the environmental variables. The occurrence (presence or absence) of roach larvae at the study sites was a binary response variable, and the site-specific potential explanatory variables (salinity, temperature, turbidity, wave exposure index (fetch), width of the reed belt, depth at the outer edge of the flattened reeds) were used as continuous variables. Data from both 2004 and 2005 were combined, yielding a total of 91 observations. A backward method was used to select variables for the model from among the main effects and the first-order interactions. A significance level of 0.05 was used as a criterion for a variable to stay in the model. The Hosmer–Lemeshow goodness-of-fit test was used to evaluate the overall fit of the model, and the classificatory power of the model was evaluated by comparing the observed and predicted responses (occurrence of roach larvae). Deviance residuals plots were used to seek influential observations, i.e., observations that might have significant impact on the model. The assumptions of logistic regression include that the observations are independent. However, in our data, 33 sites were sampled both years, causing a risk that they were correlated and not truly independent. Thus, a parallel generalized estimating equation (GEE) regression model using logit-link function was fitted for the final combination of variables. This estimates the same model as the standard logistic regression but allows dependence within clusters in the data, such as in longitudinal data. The logistic regressions were performed using the LOGISTIC procedure and the comparable GEE regression model using the GENMOD procedure of the SAS 9.1 software package (SAS Institute Inc., Cary, North Carolina).

Spatial predictive modelling

Because data analysis from 2004 and 2005 highlighted the

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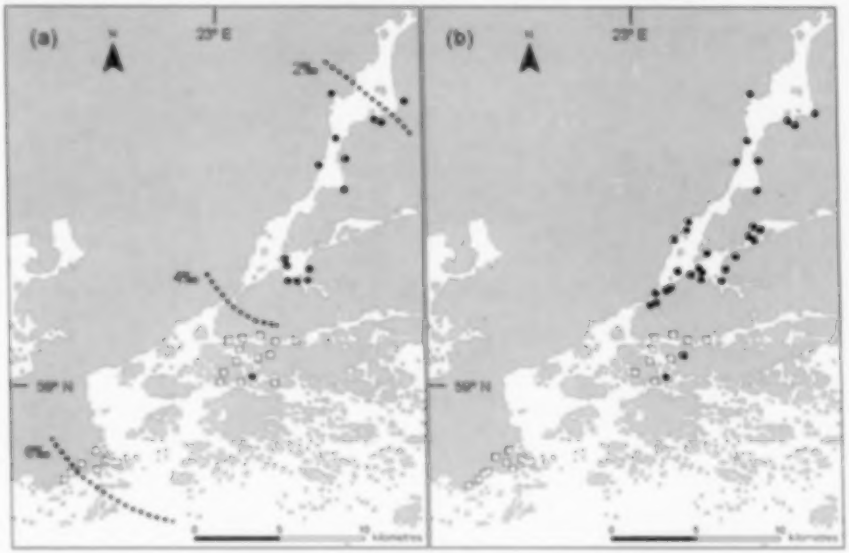
Fig. 2. The occurrence of roach larvae at the study sites in spring of (*a*) 2004 and (*b*) 2005: solid circles, sites where roach larvae were present; open squares, sites where larvae were absent. Salinity isohalines are marked with broken lines.



importance of salinity as the most significant explanatory variable, an extensive survey was executed in 2–4 May 2007, during the spawning time of roach. Salinity was measured at 144 locations in the Tammisaari archipelago to obtain environmental data for spatial modelling. The measuring points covered the entire study area with a distance of 400–2500 m between points.

The predictive spatial modelling proceeded in two steps. In the first step, the entire study area was divided into a 500 m resolution grid, and a spring surface-water salinity estimate was produced for each cell. This was carried out by importing the spatial salinity data measured in 2007 into GIS and using kriging interpolation to predict salinity for every cell from the data measured at the 144 locations. The kriging method uses variograms to express the spatial variation, and it minimizes the error of predicted values, which are estimated by spatial distribution of the predicted values (Oliver and Webster 1990). Nonparametric Spearman's correlation analysis was used to assess the performance of the salinity interpolation by comparing the interpolated and observed salinity values in the 91 sampling sites. The most representational interpolation map ($r = 0.82$) was chosen for the spatial predictive modelling purposes. In the second step, the logistic regression model and the interpolated salinity estimates were used to calculate cell-specific probabilities for the occurrence (presence or absence) of roach larvae in the reed-belt shores. Because the spawning and larval areas of roach are restricted to the littoral zone (Mills 1991) and we examined only reed-covered shores, the results on the spatial predictive map are presented only on an area close to the reed-covered shores, using a 60 m wide buffer area.

The length of the reed-belt cover along the shoreline was measured for all the 500 m × 500 m cells, based on data from aerial photos. Then, in each cell in which reed belts existed, the cell-specific probability of the presence of roach larvae was combined with the measured length of the reed-belt shore, yielding cell-specific estimates indicating the length of the reed belt covered shoreline used for roach reproduction. These values were then summed and compared with the total length of the reed-covered shoreline in the area. All GIS analyses were performed using the ArcGIS (ArcMap 9.2; ESRI, Redlands, California) software package.

Estimation of the effects of runoff on coastal salinity

Two deviant years, 2003 and 2005, were selected to describe the effects of low and high spring runoff on surface-water salinity in the study area. The runoff data from 2003 and 2005 were collected at Peltokoski sampling station, located downriver in Mustionjoki. Monthly averages of the runoff were compared with salinity values measured three times per year (March, May, July) at 12 stations situated in a line extending through our study area from the mouth of the River Mustionjoki, at the bottom of Pojo Bay, to the eastern part of Hanko Peninsula in the outer archipelago. The distances from the river mouth of the 12 sampling stations ranged from 0.5 to 34 km. Salinity measurements and runoff data were compared to determine how far from the river mouth the spring runoff water spread and affected the salinity. To describe the effect of ice cover on the spreading of runoff water and the resultant salinity, salinity was measured every two weeks at two sampling stations (17 and 24 km from the river mouth; Fig. 1) before, during, and after ice breakup, from March to July 2007.

Results

Main abiotic characteristics in the study area

A clear spatial salinity gradient was detected in the study area. The surface-water salinity was generally lowest in the innermost bay area and increased towards the intermediate and outer archipelagos (Table 1). A temporal increase in salinity during May–June was also recorded, especially in the

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Table 1. Mean (± standard error) water quality characteristics of the study sites in the innermost bay area, in the intermediate archipelago, and in the outer archipelago measured in spring from the surface water.

Variable	Innermost bay area	Intermediate archipelago	Outer archipelago
Salinity 2004 (‰)	1.9 (±0.2)	5.2 (±0.1)	6.0 (±0.1)
Salinity 2005 (‰)	1.7 (±0.1)	4.5 (±0.1)	5.3 (±0.1)
Temperature 2004 (°C)	14.5 (±0.4)	13.4 (±0.4)	11.9 (±0.4)
Temperature 2005 (°C)	13.5 (±0.2)	13.0 (±0.4)	11.9 (±0.2)
Turbidity 2004 (NTU)	4.1 (±0.2)	2.5 (±0.5)	2.4 (±0.8)
Turbidity 2005 (NTU)	4.9 (±0.5)	2.8 (±0.3)	2.7 (±0.7)
Fetch 2004 ($m^2 \cdot s^{-1}$)	4892 (±467)	2677 (±356)	3913 (±919)
Fetch 2005 ($m^2 \cdot s^{-1}$)	4748 (±266)	2500 (±321)	3842 (±791)
Reed-belt width 2004 (m)	23.7 (±3.8)	17.6 (±3.7)	10.9 (±2.3)
Reed-belt width 2005 (m)	26.2 (±3.7)	19.4 (±4.1)	10.9 (±1.9)
Depth 2004 (m)	0.7 (±0.07)	0.8 (±0.07)	0.4 (±0.07)
Depth 2005 (m)	0.7 (±0.10)	0.3 (±0.05)	0.2 (±0.05)

Note: Water depth was measured at the outer edge of the flattened reeds. NTU, nephelometric turbidity units.

inner bay area. In the spring of 2004, for instance, the mean salinity in the innermost bay area increased from 1.9‰ in early May to 3.1‰ in mid-June and in the spring of 2005 from 1.4‰ in early May to 2.3 ‰ in late May. Generally, salinity was lower in the early spring compared with during the rest of the year (Fig. 3). Spring water temperature and turbidity were generally higher in the inner bay area than in the intermediate and outer archipelagos (Table 1). A temporal increase in water temperature was also recorded: temperature in the inner bay area increased earlier in May and early June than in the outer areas. The wave exposure index (fetch) and the mean width of the reed belt and the mean water depth at the outer edge of the flattened reed zone were highest in the innermost bay area (Table 1).

Modelling the probability of roach larval presence

Roach larvae were most frequently observed in the inner bay area, where they were present at all 14 sites in 2004 and at all 29 sites in 2005 (Fig. 2). In the intermediate archipelago, roach larvae were present at 1 out of 14 sites in 2004 and at 2 out of 12 sites in 2005. In the outer archipelago, roach larvae were absent at all 10 sites in 2004 and all 12 sites in 2005.

In the final logistic regression model, the only site-specific variable explaining the occurrence of roach larvae was the spring surface-water salinity ($p < 0.001$), which was measured at the beginning of May. No other environmental variables had a significant ($p < 0.05$) explanatory effect and, therefore, did not stay in the model. The formula of logistic regression model with one explanatory factor is

$$\pi(x) = 1/(1 + e^{-(\alpha + \beta x)})$$

where, in this case, $\pi(x)$ is the probability of occurrence of roach larvae at a certain salinity (x), and α and β are estimated model parameters. The parameters (± standard error, SE) were $\alpha = 11.431 \pm 3.405$ and $\beta = -2.864 \pm 0.769$. Thus, the probability of roach larvae being present reached 50% ($\pi(x) = 0.5$) when the salinity was 3.99‰ (Fig. 4).The large p value (0.929) of the Hosmer–Lemeshow goodness-of-fit test indicated that the fitted model was very adequate. The classification table showed that the model predicted 95.6% of the observations ($n = 91$) correctly. The sensitivity was 93.5% (three false negatives), and the specificity was 97.8% (only one false positive). Deviance residuals plots highlighted three observations, which were those at which salinity was relatively high and few roach larvae were still found (see also Fig. 4), but these observations were maintained in the model. The GEE regression model produced highly similar parameter estimates ($\alpha = 11.433$ and $\beta = -2.865$) as the logistic regression model and the low estimated "working correlation" (–0.02040) indicated that the observations taken from same sites in the different years 2004 and 2005 were not correlated and, thus, could be treated as independent observations in the logistic regression models.

The potential reproduction areas of roach were concentrated in the innermost archipelago and bay area (Fig. 5). According to this spatial prediction map, based on reed-belt locations, the logistic regression model, and the salinity interpolation map combined in the GIS, roach can exploit 74% of the reed-covered shores for reproduction in the inner bay area, 3% in the intermediate archipelago, and 0.3% in the outer archipelago. Altogether, reed belts cover 27% of the total shoreline in our study area, and based on the GIS model, only 32% of these reed-covered shores are used for roach reproduction.

Factors affecting spring surface-water salinity

Freshwater runoff from the River Mustionjoki and its drainage basin reduced the surface-water salinity in the inner parts of the study area (Fig. 3). Over a distance of 35 km from the river mouth to the outer archipelago, surface-water salinity increased from 0‰ to around 6‰. When winter and springtime runoff were high, such as in 2005, the surface-water salinity during late winter was low, being close to 0‰ up to 25 km from the river mouth. In 2003, winter and spring runoff were low, and the surface-water salinity at 25 km from the river mouth was close to 4‰. Measurements taken in May were available for both years from only

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Fig. 3. The effect of freshwater runoff on spring surface-water salinity in (*a*) 2003 and (*b*) 2005. The left column presents mean monthly runoffs from the River Mustionjoki (arrows show time of ice breakup in the study area), and the right column presents the corresponding surface-water salinity values measured in March (solid triangles), May (shaded triangles), and July (open triangles) at 12 sampling stations extending from the innermost bay area to the intermediate and outer archipelagos (broken lines show critical salinity limit (4‰) for roach reproduction).

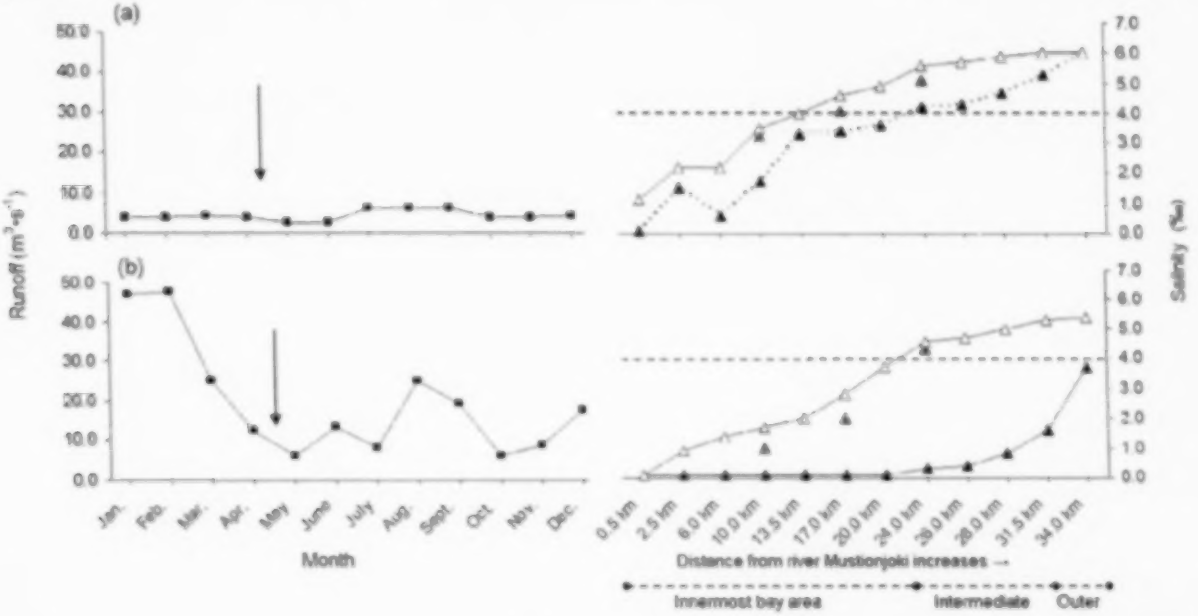
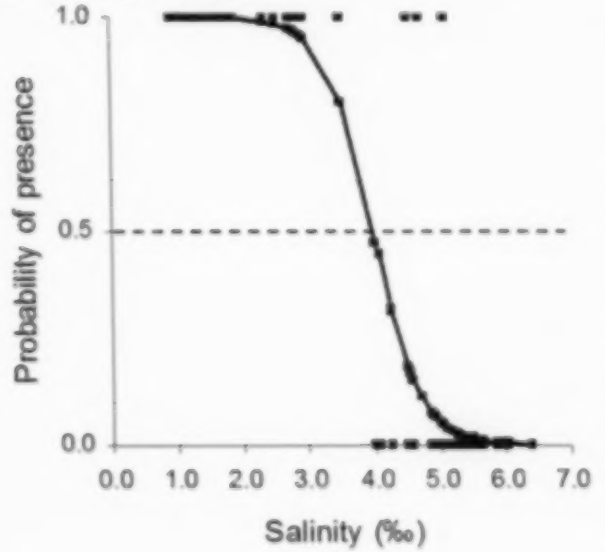


Fig. 4. Logistic regression curve explaining the effect of surface-water salinity on the occurrence of newly hatched roach larvae. The field survey observations of roach larvae (1 = present, 0 = absent) are indicated by solid squares.



three stations, but the data indicated the same pattern; during the year of high runoff (2005), the surface-water salinity was 1‰–2‰ in the innermost bay area and 4.5‰ in the intermediate archipelago, whereas during the dry year (2003), the respective figures were 3.4‰–4.1‰ and over 5‰ (Fig. 3). The area with a salinity potentially allowing roach reproduction extended 6 km further offshore in May 2005 (high runoff) than in May 2003 (low runoff) (Fig. 3). Our field survey years 2004 and 2005 were similar, with high springtime runoff conditions.

During the ice-cover period, on 13 March 2007, the surface water at the station 17 km from the river mouth, still in the inner bay area, was almost fresh, with a salinity of <0.8‰. Salinity increased to 1.1‰ at a depth of 2 m and to 1.8‰ in a depth of 4 m, close to the bottom. At the station 24 km from the river mouth and close to the border between the inner bay area and the intermediate archipelago, the salinity at the surface was <2.8‰ and increased to 3.1‰ at a depth of 2 m, to 5.3‰ at 4 m, and to 5.6‰ at 10 m. After the ice breakup, the surface-water salinity rapidly increased at both stations (Fig. 6), indicating that the winter ice cover promoted the spread of freshwater runoff over a wider area under the ice in the stratified surface-water layer and prohibited the mixing of freshwater with the more saline brackish water of the deeper layers.

Discussion

Distribution of roach reproduction areas

Our results indicate that in the northwestern part of the Gulf of Finland, roach successfully reproduce only in the inner archipelago area, where freshwater runoff has a strong influence on salinity in the spring. In the inner archipelago zone, roach reproduce on almost all vegetated shallow

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Fig. 5. Spatial predictive map showing the probability (%) of a reed-covered shore being used for roach reproduction in our study area.

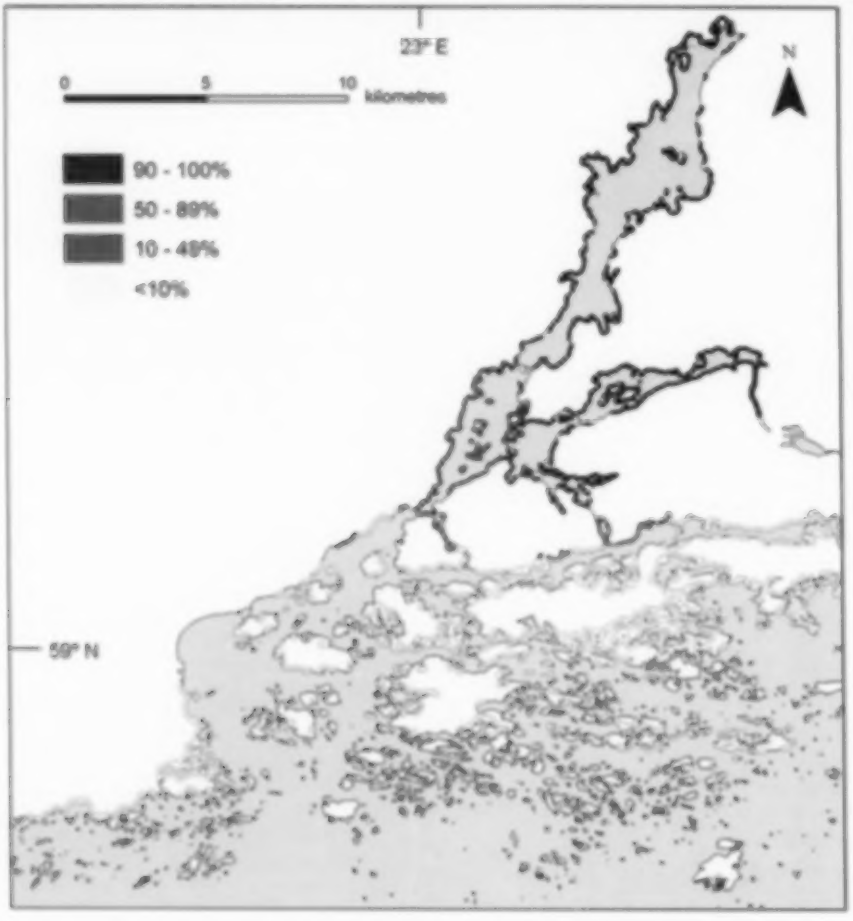
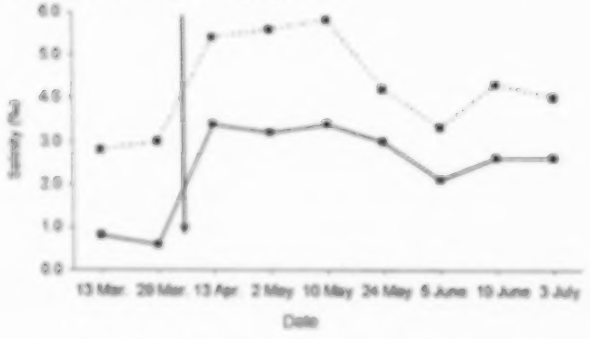


Fig. 6. The surface-water salinity in the spring of 2007 at two sampling stations, 17 km (solid line) and 24 km (broken line) from the mouth of the River Mustionjoki. The arrow indicates the time of ice breakup in the area in spring of 2007.



shores where reed belts are present and salinity in early May remains below 4‰. Our results support the laboratory studies of Jäger et al. (1981) and Klinkhardt and Winkler (1989) indicating that Baltic roach reproduction is unsuccessful in salinities exceeding 3.5‰. In the Quark area of the northern Baltic Sea, roach have also been reported to reproduce only in the coastal areas receiving substantial inputs of freshwater (Karås and Hudd 1993). According to our results, roach can also occasionally reproduce in the intermediate archipelago in the northwestern Gulf of Finland, but probably only in sheltered reed-covered inlets where local freshwater sources, such as small ditches, reduce the salinity and water exchange is low. Sensitivity to salinity makes the roach more confined to the inner bay areas than many other freshwater fish. Lappalainen et al. (2008) have recently shown that in the same area, pike (*Esox lucius*), another freshwater fish species, is able to use reed-belt shores for reproduction even in the outer archipelago.

In our model, salinity was the only significant environmental variable explaining the occurrence of roach larvae at the study sites. Of the other measured environmental variables, water temperature could potentially limit roach reproduction areas (Černý 1974), but in our study area, the average water temperatures in all subareas exceeded the critical level (9 °C) reported by Černý (1974) during the time of reproduction. In our data, both turbidity and temperature correlated negatively with salinity. Turbidity is at least partly linked to eutrophication, and eutrophic inner bay

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areas can promote the survival and production of juvenile roach by increasing the supply of suitable habitats with abundant zooplankton food. Characteristics of the reed-belt structure, such as the width of the reed belt, did not limit the reproduction areas of roach, at least not in this model, as roach reproduced in the inner bay area on almost all reed-belt shores that were identified from aerial photos. The wave exposure index (fetch) did not show any explanatory power in the model either.

Effects of climate change on the extent of roach reproduction areas

Low salinity in the spring is important for successful roach reproduction. Climate change could affect the spring salinity in potential coastal roach reproduction areas via at least three mechanisms. (*i*) The average salinity of the Baltic Sea open-water area is predicted to decrease between 8% and 50% by the late 21st century because of an increase in the total freshwater supply to the Baltic Sea (Meier 2006; Meier et al. 2006). The absence of major intrusions of saline water since the 1980s has also contributed to the salinity changes (Hänninen et al. 2000). On the other hand, climate change also will have other consequences that are partially contradictory to the first one. (*ii*) More freshwater will enter the coastal area, but the peak of freshwater runoff may shift from the spring to the winter, preceding the spawning time of roach. According to recent climate change scenarios, the amount of winter rainfall is projected to increase and winter freshwater runoff during the warmer winters is therefore expected to increase considerably in the coastal areas of the northern Baltic Sea (Carter et al. 2005; Silander et al. 2006). With more precipitation and runoff in winter and less snow, there will be less spring runoff from melting snow at the time of roach reproduction. This means that the spreading of freshwater runoff in spring far from river mouths, which takes place at present and was demonstrated with our data, could weaken and occur earlier. (*iii*) The coastal areas of the northern Baltic Sea are typically ice-covered in winter. The winter ice cover affects the dispersal of freshwater runoff from rivers in coastal waters and has a clear effect on salinity and water quality. This is especially apparent in winter and early spring, when river water travels very long distances as a thin layer (1–2 m) under the ice, as we showed (see also Paavilainen et al. 1985). During the open-water period, the area affected by river runoff water is more restricted. However, the duration of the sea ice period is decreasing in the northern Baltic Sea and there is a trend towards earlier ice breakup (Seinä et al. 1996, 2001; Jevrejeva et al. 2004). This could limit the width of the coastal zone receiving substantial amounts of freshwater. Of course, roach reproduction consequently also may take place earlier in the spring. If the sea ice period decreases to only a couple of months during midwinter, it is likely that the spreading of freshwater runoff will diminish during the roach reproduction period.

The most likely consequence, however, is that the predicted general reduction of Baltic Sea salinity will overwhelm the two rather temporal and local mechanisms (*ii* and *iii*), and the likely outcome is therefore reduced spring salinity in the coastal areas. If the surface-water salinity during the reproduction time considerably decreases, roach reproduction areas will most likely expand to reed-belt shores in areas where salinity is currently too high for reproduction. In our study area, assuming that the extent of reed belts remains at the present level, that all of them are suitable spawning and larval grounds for roach, and that the salinity in the whole area remains below 4‰, the coastal shoreline suitable for roach reproduction could increase at least threefold, as 68% of the reed-covered shores are currently unsuitable for reproduction. Similar reed-belt shores with salinity higher than 4‰ are also common in other archipelago areas in the northern Baltic Sea and, therefore, also represent potential future reproduction areas for roach. This pattern matches well with the studies of Mackenzie et al. (2007), who have stated that the straightforward consequence of the Baltic environment changing towards less saline conditions is that coastal freshwater fish species will be able to enlarge their distribution.

An increasing abundance of roach could have widespread ecosystem effects. The ecosystem effects of dense roach populations have been well demonstrated in eutrophic lakes, and it has been shown that the mass removal of roach and other cyprinids can be used to biomanipulate the food web by decreasing the predation pressure on zooplankton and furthermore to improve water quality (e.g., Shapiro et al. 1975; Drenner and Hambright 1999; Kairesalo et al. 1999). In the magnitude of the Baltic Sea, the consequence of the increasing roach population is, however, not this simple, but some effects are already now evident. In the Baltic coastal area, the feeding areas of adult roach also cover the outer archipelago, and an increased abundance of roach with their high predation pressure negatively affects the populations of blue mussel (*Mytilus edulis*) (Lappalainen et al. 2005; Westerbom et al. 2006). The enormous predation pressure of Caspian roach on molluscs has also been demonstrated in the northern Caspian Sea (Zheltenkova 1939, 1951; Belova 1986).

Evaluation of the mapping methods

Several statistical techniques and GIS tools are available to model and predict the spatial distribution of species based on environmental variables. Recently, an increasing number of predictive models have been used in aquatic ecology to predict, for example, the distribution of seagrass beds (Kelly et al. 2001; Greve and Krause-Jensen 2005) and macrophytes (Lehmann 1998), the spawning habitat of sole (*Solea solea*) (Eastwood et al. 2001) and sandeel (*Ammodytes marinus*) (Wright et al. 2000), and the presence and abundance of small fish (Francis et al. 2005) and intertidal communities (Zacharias et al. 1999). Here we fitted a logistic regression model, allowing the use of several explanatory environmental variables. Such regression models are very popular and often used for modelling species distributions (Guisan and Zimmermann 2000). This approach was appropriate here because salinity proved to be a strong explanatory factor and the reproduction areas of roach were relatively straightforward to predict. When more complex combinations of weaker explanatory variables are used to predict species distribution, more complex and flexible models are needed. For example, generalized additive models (GAM), which are an extension of generalized linear models (GLM), have the advantage of better handling highly nonlinear relationships that

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are common in nature (Guisan et al. 2002). In spatial predictive modelling, regardless of the modelling technique, it is important that the sampling sites are evenly distributed and thus cover large parts of the environmental gradient that determines the distribution of the target species (Guisan and Zimmermann 2000). In this study, the entire salinity gradient was covered in the random-stratified field sampling. Salinity together with reed belts were assumed to be directly responsible for the distribution of roach reproduction habitats in our study area. This model was constructed to map the potential roach reproduction areas in the northwestern Gulf of Finland. It can be assumed that regional differences in the roach–environment relationship are small in the northern Baltic Sea. Therefore, this spatial predictive model for roach could be applied to other coastal areas in the northern Baltic Sea.

Spatial prediction maps have the potential to develop into a valuable tool in the large-scale mapping of fish reproduction areas. There is undoubtedly a need for information on the reproduction areas of fish, and this type of information will help promote the sustainable use of coastal areas. When presenting a spatial modelling outcome, detailed continuous high-resolution maps of the explanatory variables are needed. We mapped the reed-belt locations using aerial photographs, which proved to be accurate and have a high spatial resolution, but the visual interpretation was laborious and expensive as a large-scale mapping method. Therefore, the next step and a more efficient approach to attain large-scale maps of reed-belt locations could be to use satellite imagery such as Landsat TM/ETM+ frames (Pitkänen et al. 2007) and supervised computer-based interpretation.

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Relating crustacean zooplankton community structure to residential development and land-cover disturbance near Canadian Shield lakes

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Abstract: We applied the land-cover cascade (LCC) concept to evaluate whether human disturbance affects crustacean zooplankton community in 13 nutrient-poor lakes of the Laurentian region along a gradient of residential development and land-cover disturbance. Zooplankton biomass responded indirectly to residential disturbances within the watershed based on path analysis. In most LCC models, total phosphorus enrichment was a strong intermediate element linking residential disturbances to increased biomass of small crustaceans (*Bosmina*, *Ceriodaphnia*, and *Diaphanosoma*) and *Daphnia* species (*D. g. mendotae*, *D. dubia*, and *D. ambigua*). Zooplanktivory index was also an intermediate element in LCC models of crustacean functional groups and an independent element in daphnid-based LCC models. Low zooplanktivory was associated with higher biomass of large *Daphnia* (*D. pulicaria* and *D. catawba*). However, residential disturbance and select environmental factors (phosphate enrichment, zooplanktivory index, lake depth, littoral macrophytes) explained only 42% and 57%, respectively, of among-lake variation in crustacean community and *Daphnia* assemblages. Variation partitioning analysis suggested that planktivory had a similar or greater influence than human disturbance and phosphate enrichment. Our study improves the knowledge of crustacean zooplankton responses to increasing residential development and land-use disturbances near Canadian Shield lakes.

Résumé : Nous avons utilisé le concept de la cascade (LCC, « land-cover cascade ») pour évaluer si les perturbations humaines affectent la communauté de crustacés planctoniques dans 13 lacs des Laurentides, encore pauvres en nutriments, le long d'un gradient de développement résidentiel et de déboisement dans le bassin versant. L'analyse de pistes (« path analysis ») montre que les changements dans la biomasse des crustacés planctoniques reflétaient surtout le niveau de développement résidentiel dans l'ensemble du bassin versant. Dans la plupart des modèles LCC, l'enrichissement en phosphore total jouait un rôle intermédiaire essentiel reliant les perturbations aux hausses de biomasse des petits crustacés (*Bosmina*, *Ceriodaphnia* et *Diaphanosoma*) et des petites espèces de *Daphnia* (*D. g. mendotae*, *D. dubia* et *D. ambigua*). L'indice de planctivorie était aussi un l'élément intermédiaire dans les modèles LCC des groupes fonctionnels de crustacés, et un élément indépendant dans ceux des espèces de *Daphnia*. Une plus faible planctivorie était associée à la présence des grandes espèces de *Daphnia* (*D. pulicaria* et *D. catawba*). Cependant, seulement 42 % et 57 % des variations inter-lacs dans la communauté des crustacés et les regroupements d'espèces de *Daphnia* étaient expliqués respectivement par le niveau de développement résidentiel dans le bassin versant et les facteurs environnementaux choisis (enrichissement en phosphore, indice de planctivorie, profondeur du lac, abondance des macrophytes). De plus, l'analyse de partition de la variance indique que le niveau de planctivorie avait une influence plus grande ou similaire à celles du développement résidentiel et de l'enrichissement en phosphates. Notre étude permet une meilleure compréhension des changements de la communauté zooplanctonique dans les lacs du Bouclier Canadien faisant face à des perturbations suite au développement résidentiel et à l'utilisation anthropique du territoire.

Introduction

Identifying the effects of residential and land-cover disturbances within lakes and watersheds on aquatic biota is crucial for the development of an ecological management framework coupling terrestrial and aquatic environments. The land-cover cascade (LCC) concept was recently introduced as an organized approach to quantify the transfer of land-cover disturbance effects to stream biota (Burcher et al. 2007), suggesting important indirect links between land use and community responses. By identifying key intermediate components, the LCC concept determines pathways and mechanisms linking human disturbances to changes in the biota. The LCC concept has not yet been applied to detect land-cover disturbance effects on lacustrine biota.

Previous studies that related shore and watershed land use to changes in abiotic and biotic features of lakes focused on water nutrient enrichment and changes in microbial food

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Table 1. Morphometric characteristics and indices of watersheds and lakes.

Lakes	Codes	WA (km²)	Slope (%)	Rd	Rdv	LA (km²)	Volume (m³)	Lake depth		
								Z_{max} (m)	Z_{mean} (m)	Z_{rel}
Cabane	Cab	2.45	7.5	8.8	1.34	0.25	1 836 000	20.8	7.3	3.13
Croche	Cro	0.84	13	3.7	0.96	0.18	877 000	12.9	4.9	2.69
Violon	Vio	4.41	9.2	10.5	1.33	0.38	3 325 000	22.6	8.9	2.63
Blanche	Bla	3.94	8.2	8.6	0.86	0.41	4 590 000	26	11.7	3.59
du Nord	Nor	13.94	12.5	15.1	2.53	0.87	5 514 000	20.6	6.4	1.74
Gervais	Ger	8.65	6.2	8	0.37	0.97	23 526 000	60	24.4	5.41
Connelly	Con	24.36	6.2	18.6	2.55	1.24	9 561 000	20.8	7.7	1.65
Morency	Mor	2.33	14.6	8	1.04	0.26	2 246 000	20.3	8.7	3.53
Purvis	Pur	0.6	10.6	2.2	0.42	0.19	1 446 000	19.7	7.8	4
Truite	Tru	4.23	5.9	7.3	0.89	0.51	4 783 000	21.6	9.3	2.67
Tracy	Tra	0.24	8.3	1.9	0.36	0.08	676 000	22.9	8.1	7.03
Renée	Ren	0.2	10.1	2.1	0.73	0.07	296 000	9.7	4.2	3.23
Rond	Ron	1.5	7.5	8	1.24	0.17	1 206 000	15.9	7.2	3.45
Minimum		0.2	5.9	1.9	0.36	0.07	296 000	9.7	4.2	1.65
Maximum		24.36	14.6	18.6	2.55	1.24	23 526 000	60	24.4	7.03
Mean		5.21	9.21	7.91	1.12	0.43	4 606 307	22.6	8.96	3.44

Note: WA, watershed area; slope, watershed mean slope; Rd, ratio of drainage to lake area; Rdv, ratio of drainage to lake volume; LA, lake area; volume, lake volume; Z_{max}, maximum lake depth; Z_{mean}, mean lake depth; Z_{rel}, relative lake depth.

web and plankton communities (Dodson et al. 2005; Hoffmann and Dodson 2005; Burns and Galbraith 2007). In north-temperate lakes, nutrient enrichment resulting from watershed and lakeshore land use increased phytoplankton biomass (Dillon and Rigler 1974; Knoll et al. 2003) and altered zooplankton species richness and community structure (Pace 1986; Stemberger and Lazorchak 1994; Dodson et al. 2005). However, none of these studies adopted the LCC concept to evaluate both direct and indirect effects of human disturbances within lake watersheds on zooplankton communities.

Zooplankton communities provide an ideal system for studying the effects of anthropogenic land use (Dodson et al. 2005). They are considered good indicators of human disturbances for use in regional monitoring programs of lakes (Stemberger et al. 2001). Crustacean biomass and community structure change with lake trophic status at regional and continental scales and total phosphorus can be a better predictors of changes in crustacean biomass than chlorophyll *a* (Chl *a*) (Pace 1986; Yan 1986; Pinto-Coelho et al. 2005). Zooplankton are sensitive to large-scale watershed disturbances caused by agriculture (Stemberger and Lazorchak 1994; Dodson et al. 2005, 2007) and forest logging and wildfires (Patoine et al. 2000, 2002). However, zooplankton responses to small-scale disturbances by residential development and land-cover clearing of relatively pristine lake watersheds have not been assessed. Understanding the effects of human disturbances within lake watersheds on crustacean herbivores is crucial for predicting changes in water quality and phytoplankton blooms in nutrient-poor Canadian Shield lakes, because cladocerans and especially *Daphnia* can control water clarity and algal biomass (Mazumder 1994; Mazumder and Havens 1998; Stemberger and Miller 2003).

The current study is the first attempt to detect the influence of recent residential development and land-cover disturbance on crustacean zooplankton in 13 nutrient-poor lakes of the Laurentian region (Québec, Canada). We compared the relative influence of variables associated with (*i*) residential development and land-cover disturbances, (*ii*) changes in water chemistry and algal concentration, zooplanktivory, and macrophyte development, and (*iii*) natural variation in morphometry of lakes and watersheds. Firstly, we hypothesized that total biomass of crustaceans would increase along the gradient of residential and land-cover disturbance and that this increase would be more directly related to nutrient enrichment than to increasing algal biomass, as previously observed in other studies (Yan 1986; Pinto-Coelho et al. 2005). We also hypothesized that residential development and land-cover disturbance would affect differently crustacean groups and taxa. Thus, we applied the LCC approach (Burcher et al. 2007) to detect cascading pathways linking human disturbances within the watershed to variation in the biomass of crustacean taxa and *Daphnia* species. This method enabled us to test the direct and indirect influence of either residential development or land-cover clearing within the watershed through intermediate increases in nutrients and algal biomass on the biomass of each crustacean functional group and *Daphnia* species. However, as nutrient and algal biomass in Canadian Shield lakes tend to be higher in lakes with high drainage ratios and flat watershed slope (D'Arcy and Carignan 1997), changes in zooplankton biomass may be indirectly influenced by the morphometry of the lakes and watersheds. Furthermore, zooplanktivory pressure and the presence of refuge in deep zones and littoral macrophytes may also greatly influence *Daphnia* species, the major prey of young fish (Gélinas et al. 2007). Finally, using multivariate analysis, we evaluated the potential of crustacean functional groups and *Daphnia* species as bioindicators of human disturbances in watersheds relative to changes in other environmental factors.

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Materials and methods

Study sites

Sampling was conducted in 13 lakes of the Laurentian region, north of Montréal (Canada), within a 65 km radius of the Station de Biologie des Laurentides (SBL; 45°59′N, 73°60′W). The Laurentian region has supported one of the most important demographic developments in the province of Quebec since the 1970s, with a 98% rise in its residential population, while changes to agricultural and industrial activities remain negligible (Laurin 2000; Institut de la Statistique du Québec, www.stat.gouv.qc.ca). The Laurentian region is of glacial origin (Wisconsin glaciation, 18 000 years ago) and is characterized by a continental climate. The mean annual temperature varies around 4 °C, and the mean annual precipitation varies between 1100 and 1400 mm (25%–40% as snow) (Savage 2001). The bedrock is composed predominantly of granitic and gneiss rocks. The landscape is carved into hills and valleys. Forests are dominated by yellow birch (*Betula alleghaniensis*), sugar maple (*Acer saccharum*), balsam fir (*Abies balsamea*), trembling aspen (*Populus tremuloides*), and black spruce (*Picea mariana*) (Savage 2001). All lakes contained planktivorous fish (minnows (*Cyprinidae*), pumpkinseed sunfish (*Lepomis gibbosus*), rock bass (*Amblophites rupestris*); D. Boisclair, Université de Montréal, Département de Sciences Biologiques, Montréal, Québec, Canada, personal communication) but were stocked at some point (records started in 1944) with piscivorous and (or) benthivorous fish, mostly brook trout (*Salvelinus fontinalis*), rainbow trout (*Oncorhynchus mykiss*), and walleye (*Stizostedion vitreum*).

Lakes selected for this study were chosen to represent the wide, natural diversity in lake and watershed morphometry and in water chemistry and the varying trophic states of lakes that occur in the Laurentian region, while ranging along a gradient in residential development and land-cover disturbance. These lakes covered a large range of waterbody volume and catchment areas and of drainage ratio; lake depth also varied sixfold (Table 1). According to trophic classification (Organisation de Coopération et de Développement Économiques (OCDE) 1982), the lakes were oligotrophic, except for three lakes (René, Rond, and Connelly), which reached mesotrophic states, with mean summer nutrient concentrations exceeding 10 µg total phosphorus (TP)·L^{-1} or 340 µg total nitrogen (TN)·L^{-1} and 3.8 µg Chl *a*·L^{-1} (Table 2). The lakes also covered a wide range in Secchi transparency, euphotic depth, and dissolved organic carbon (DOC) concentrations (Table 2). Most of the lakes were circumneutral or slightly acidic, except for two (Morency and Rond), which were surrounded by important recreational infrastructures and roads that were slightly alkaline (Table 2). Gradients in residential development and land-cover disturbance ranged from 0 to 340 dwellings·km^{-2} and from 0% to 53% of cleared forest (open area) within the watershed (Table 3). The intensity of the disturbance was the highest nearshore, with maxima of 686 dwellings·km^{-2} and 86% of cleared forest occurring within the 50 m riparian zone (Tables 1, 2, and 3).

Morphometry of lakes and watersheds and disturbance gradients

Morphometric characteristics of watersheds (area and

Table 2. Chlorophyll *a* biomass, water chemistry (TP, TN, pH, DOC), Secchi transparency, euphotic zone depth, macrophyte biomass, and body length metrics (cladocerans and *Daphnia*) for the 13 studied lakes.

Lakes	Codes	Chl *a* (µg·L^{-1})	TP (µg·L^{-1})	TN (µg·L^{-1})	pH	DOC (mg·L^{-1})	Secchi (m)	EZ (m)	Macro (g·m^{-2})	BLM-CL (mm)	BLM-D (mm)
Cabane	Cab	3.0±0.7	8.2±2.1	259.8±20.6	6.2	3.6±0.0	3.3±0.3	7.1±0.7	0.011	0.769	0.832
Croche	Cro	1.2±0.9	5.0±0.3	256.9±23.1	6.2	4.1±0.3	4.0±0.2	6.8±0.2	0.011	0.965	1.027
Vinbon	Vin	1.2±0.1	5.7±1.3	215.1±16.1	6.6	3.5±0.3	5.3±1.0	7.5±0.5	3.055	0.914	1.140
Blanche	Bla	2.6±0.9	4.9±0.3	258.0±10.9	6.4	2.8±0.1	5.3±0.1	11.8±1.1	0.0611	0.848	0.998
du Nord	Nor	1.5±0.9	9.9±2.1	329.2±40.1	6.7	6.2±0.3	3.2±0.2	5.6±0.2	1.183	0.611	0.871
Gervais	Ger	0.7±0.4	4.6±0.6	190.4±12.8	7	2.7±0.2	7.7±0.5	12.3±3.7	0.011	1.192	1.235
Connelly	Con	4.4±4.4	8.0±0.4	340.0±38.6	7.4	4.6±0.03	3.5±0.7	7.0±0.5	25.277	0.740	0.816
Morency	Mor	2.3±0.8	10.4±0.8	313.6±3.3	7.8	3.3±0.2	4.7±1.0	8.8±0.2	2.044	0.835	1.106
Parvis	Par	2.7±1.4	10.3±1.9	272.7±13.7	7.2	3.1±0.1	6.2±1.5	12.0±1.3	3.116	0.831	0.972
Triste	Tri	1.7±1.2	6.5±1.7	280.5±18.5	7.7	3.0±0.1	5.2±1.1	11.0±0.8	11.067	0.934	1.007
Tracy	Tra	1.3±1.1	6.2±0.4	266.5±87.2	7	2.9±0.2	5.3±1.7	11.1±1.2	0.011	1.281	1.415
Renzo	Ren	3.8±1.7	14.2±2.1	485.6±67.2	6.1	4.2±0.1	4.0±1.8	8.6±2.4	5.116	0.545	0.659
Rond	Ron	4.6±5.8	13.0±4.8	384.6±140	8.1	3.6±0.2	5.5±1.3	10.1±2.5	24.427	0.811	1.007
Minimum		0.7	4.6	190.4	6.1	2.7	3.2	5.6	0.01	0.54	0.65
Maximum		4.6	14.2	485.6	8.1	6.2	7.7	12.3	25.27	1.28	1.41
Mean		2.38	8.22	297.15	6.95	3.66	4.83	9.21	5.79	0.87	1.01

Notes: Values represent means ± standard errors during summer 2003, except for the macrophyte biomass (median). Chl *a*, chlorophyll *a*; TP, total phosphorus; TN, total nitrogen; DOC, dissolved organic carbon; EZ, euphotic zone depth; macro, macrophyte biomass; BLM-CL, body length metrics (cladocerans); BLM-D, body length metrics (*Daphnia*).

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Table 3. Residential development (RD) and land-cover disturbance (LCD) within the watershed and nearshore riparian strips.

		Residential development (no. of dwellings·km^{-2})								LCD (% of open area)	
Lakes	Codes	50 m (RD50)	100 m (RD100)	150 m (RD150)	200 m (RD200)	250 m (RD250)	500 m (RD500)	1000 m (RD1000)	Watershed (RDW)	50 m (LC50)	Watershed (LCW)
Cabane	Cab	0	0	0	0	0	0	0	0	0	0
Croche	Cro	5	3	2	2	2	2	2	2	0	0
Violon	Vio	8	13	9	7	5	5	3	3	0	0
Blanche	Bla	219	123	89	73	63	47	26	16	17	1
du Nord	Nor	261	167	127	100	82	44	23	18	40	3
Gervais	Ger	212	115	82	66	56	33	27	21	2	0.4
Connelly	Con	686	552	448	389	339	217	157	48	46	7
Morency	Mor	152	151	126	116	103	69	57	57	31	13
Purvis	Pur	106	117	100	101	94	89	89	89	13	8
Truite	Tru	430	400	404	368	322	202	123	111	79	30
Tracy	Tra	289	176	157	144	144	144	144	144	5	4
Renée	Ren	388	400	406	393	381	340	340	340	38	30
Rond	Ron	323	373	417	400	391	330	255	242	86	53
Minimum		0	0	0	0	0	0	0	0	0	0
Maximum		686	552	448	400	391	340	340	340	86	53
Mean		236.84	199.23	182.07	166.07	152.46	117.07	95.84	83.92	27.46	11.49

Note: RD in riparian strips of increasing widths: 50 m, RD50; 100 m, RD100; 150 m, RD150; 200 m, RD200; 250 m, RD250; 500 m, RD500; 1000 m, RD1000; RDW, entire watershed.

slope) and lakes (area, volume, and maximum and mean depth) were measured (MapInfo, version 6.5; Pitney Bowes Software Inc., Troy, New York) from numerical topographic and bathymetric maps (Lambert et al. 2008). From these variables, we calculated several morphological indices for watersheds (ratio of drainage to lake area or volume) and lakes (relative depth). To evaluate human disturbances by residential development (RD) and land-cover disturbance (LCD), we estimated the density of dwellings (number of dwellings·km^{-2}) and the percentage of open area on orthorectified aerial photographs (1 : 30 000 and 1 : 10 000) acquired in 2002 and 2004 (MapInfo, version 6.5). Because residential development in the Laurentian region is usually concentrated around lake shores, we measured dwelling density at subwatershed scales (within riparian strips of increasing width: 50, 100, 150, 200, 250, 500, and 1000 m), as well as within the entire watershed (Table 3). LCD was estimated as the percentage of open area within the nearshore riparian zone (within 50 m) and within the entire watershed. Open areas represented previously forested zones cleared for buildings, roads, parking lots, and lawns; a golf course and downhill ski trails were additional features near two lakes. Macrophyte coverage in the littoral zone was roughly estimated during July at a depth of approximately 1 m in three quadrats at three sites in each lake; the median value was used as an index of macrophyte biomass because of the high variation between quadrats (A. Cattaneo, Université de Montréal, Département de Sciences Biologiques, Montréal, Québec, Canada, unpublished data).

Water sampling and analysis

Sampling was carried out in June, July, and August of 2003 at the location of maximum depth in each lake to reflect the influence of human disturbances on water quality in the pelagic zone. Temperature, oxygen concentration, and pH were measured at 1 m intervals through the water column with a Hydrolab Surveyor® 4 (Hach Company, Loveland, Colorado), whereas light intensity was measured with a LI-1000 photometer (LI-COR Biosciences, Lincoln, Nebraska). Secchi depth transparency was measured, and the euphotic zone (EZ) was estimated as the depth of 1% of surface light irradiance. Euphotic depth-integrated water samples were collected with weighted Tygon™ tubing fitted with a one-way foot valve. Water samples were transferred into two 1 L polypropylene containers, a dark one for chl *a* analysis and a clear one for the measurement of total phosphorus (TP), total dissolved phosphorus (TDP), total nitrogen (TN), dissolved organic carbon (DOC), and pH. In the laboratory, water samples were filtered on GF/C Whatman filters (1.2 µm pore size), and the filters were kept frozen until analysis for Chl *a*. Phytoplankton pigments were extracted in cold 95% ethanol for 24 h and measured before and after acidification at 665 nm and 750 nm (Spectronic Genesys 5 spectrophotometer; Thermo Fisher Scientific Inc., Waltham, Mass.) (Nusch 1980). TP and TDP concentrations were determined on 50 mL of unfiltered and filtered (Gelman type glass-fibre filters, 0.45 µm pore size) water samples, respectively. TP and TDP samples were analysed as orthophosphate after persulfate digestion in an autoclave (Stainton et al. 1977). TN was analysed with a continuous-flow QuickChem 8000 (Lachat Instruments, Hach Company), and DOC was analysed with an ASI-5000A (Shimadzu Corporation, Kyoto, Japan).

Zooplankton sampling and analysis

Zooplankton was also sampled in June, July, and August of 2003 with a conical plankton net (12 cm aperture and 153 µm mesh size) in the deepest site in each lake using vertical hauls from 1 m above the sediments to lake surface. Samples were preserved in buffered 4% sucrose–formalin after narcotisation in carbonated water. Taxonomic enumerations, identification, and size measurements were done on 10 mL subsamples withdrawn using a large pipette from entire concentrated samples that had been gently mixed. For the direct sizing of organisms, subsamples were poured into

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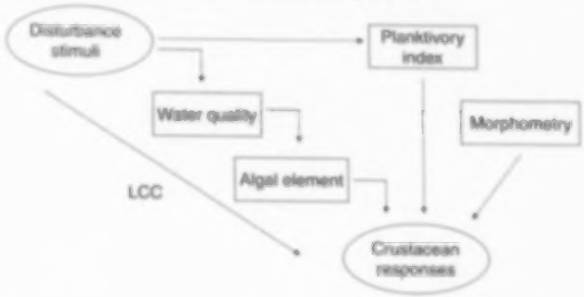
a grooved Plexiglas Ward counting cell (10 mL capacity) and placed under a dissecting microscope (Leica MZ20) that was connected to an image analyser (Image Pro Plus, Media Cybernetics Inc., Bethesda, Maryland). Copepod nauplii and rotifers were excluded from the analysis because the 153 µm mesh size plankton net did not adequately collect nauplii stages and small rotifers; it collected only the large rotifer *Asplanchna* and species with spines, such as *Keratella* and *Kellicottia*. Body lengths of cladocerans were measured from the top of the head to the base of the tail spine. Copepods were measured from the top of the head to the end of the caudal ramus. Generally, a minimum of 20 individuals were measured for each crustacean functional group, and a minimum of 100 individuals were measured for each *Daphnia* species, or all individuals present in the entire sample were measured if these minimums could not be obtained from the 10 mL subsample. To estimate the biomass of each crustacean functional group or *Daphnia* species (see below), body lengths were converted to dry weights using published regression models (Malley et al. 1989).

Crustaceans were classified into functional groups according to their feeding mode and body size. This classification into functional groups implied that co-members of any group played a similar role in the ecosystem (Stemberger and Lazorchak 1994). Cladocerans were divided into different feeding groups: filter feeders, filter and grasping feeders, and raptorial feeders. Large cladocerans such as *Daphnia*, *Ceriodaphnia*, *Diaphanosoma*, and *Holopedium* were considered as filter feeders because they can filter a broad size range of particles, with maximum particle size usually increasing with herbivore body length (Chow-Fraser and Knoechel 1985; Knoechel and Holtby 1986). Small cladocerans such as *Bosmina* use distinct feeding modes by combining the use of both filter and grasp feeding techniques on bacteria, small algae, and flagellates (DeMott and Kerfoot 1982; Vanderploeg 1990). The filtration rates for copepods are less efficient than those of cladocerans of similar size, and they select large particles (Peters and Downing 1984). Copepods were divided into two feeding groups: the calanoids, which collect a variety of small and large particles using passive and active mechanisms (Vanderploeg 1990), and the cyclopoids, which are raptorial omnivores and effective predators also feeding on algae (Adrian and Frost 1993). Therefore, according to our classification, crustaceans were separated into seven functional groups: calanoids, cyclopoids, *Daphnia*, *Ceriodaphnia*, *Holopedium*, *Diaphanosoma*, and *Bosmina*. *Daphnia* species were further considered separately because they present different responses to planktivory depending of their size (Gélinas et al. 2007) and because competition and grazer–resource interactions vary among different-sized *Daphnia* species (Kasprzak et al. 1999; Kreutzer and Lampert 1999; Tessier et al. 2001).

To roughly estimate zooplanktivory pressure, we calculated body length metrics based on the mean of the 50 individuals measured in each crustacean functional group in each lake. Their density was not taken into account to remove the possible bias due to high abundance of dominant *Daphnia* in some lakes (Table 2). Although not perfect, such an index might give us some indication of relative zooplanktivory pressure by young fish and invertebrate predators in each lake (Brooks and Dodson 1965).

Fig. 1. Conceptual land-cover cascade (LCC) model linking watershed disturbance to crustacean zooplankton responses via intermediate (water quality, algal biomass, zooplanktivory index) and independent (morphometry) elements. Path arrows represent mechanistic cause–effect links between disturbance stimuli, elements, and crustacean zooplankton responses.



Statistical analyses

To study the relationships among all measured variables in each lake, a principal component analysis (PCA) was performed, incorporating all measured disturbance and environmental factors: watershed and lake morphometry (Table 1), water quality (Table 2), and disturbance variables (Table 3). PCA was performed using CANOCO version 4.0 (ter Braak 1988, 1990). Simple linear regression models based on summer means served to identify underlying relationships between disturbance factors, water quality, algal biomass, and the biomass of total crustaceans and each functional group. Only significant regression models were retained after Bonferroni adjustments. No transformation was needed, the data were normally distributed ($P > 0.05$; Kolmogorov–Smirnov test), and all univariate statistical analyses were conducted using Systat 8 (Systat Software Inc., Point Richmond, California).

As the land-cover cascade (LCC) concept implies that land-cover disturbance and biota interact indirectly through one or several intermediate elements or links (Burcher et al. 2007), we built a conceptual LCC design (Fig. 1) to address both direct and indirect pathways between residential and land-cover disturbances and environmental factors and the biomass of crustacean functional groups and *Daphnia* species. We conducted path analysis (Shipley 2000) to identify how residential and land-cover disturbance within the watershed might be transferred to crustacean zooplankton biota in lakes. The possible key intermediate elements were nutrient and algal enrichment, zooplanktivory index, and lake and watershed morphology. We arranged conceivable paths to test if our data fit hypothesized LCC paths between disturbance factors and changes in biomass of crustacean functional groups and *Daphnia* species. Model fit was determined primarily by mean squared error approximation (RMSEA), where a significant RMSEA ($P < 0.05$) indicated no significant difference between the data and our LCC models. Path analyses were performed using Systat version 8 (Wilkinson and the Task Force on Statistical Inference 1999). In addition, path coefficients were calculated corresponding to

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bivariate correlation coefficients and quantifying the strength and direction of the intermediate links between disturbance and environmental factors and crustacean zooplankton responses, overall, characterizing existing cascades.

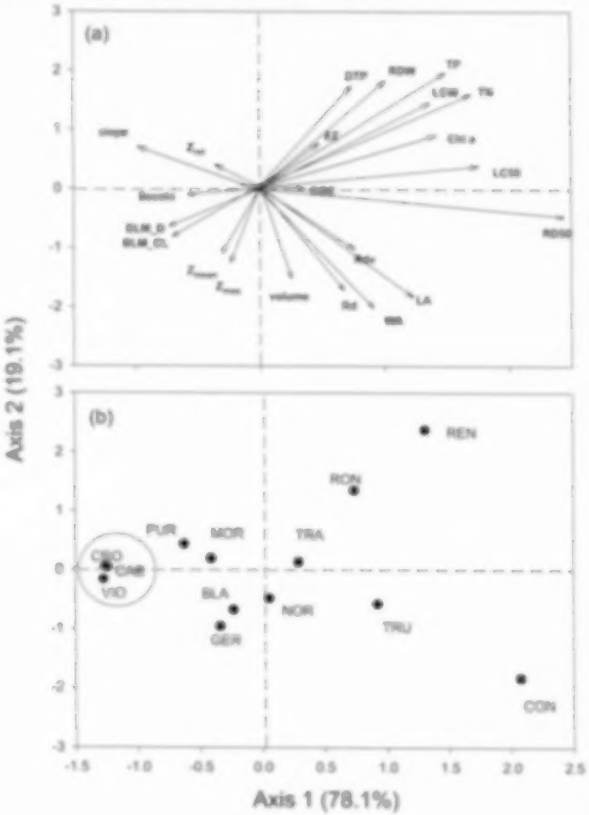
To determine if crustacean functional groups and *Daphnia* species could serve as bioindicators of human disturbances in lake watersheds, redundancy analyses (RDAs) were performed to assess changes in crustacean community structure and *Daphnia* species assemblages in response to the main disturbance gradients, as well as to environmental factors that described the lacustrine environments (Legendre and Legendre 1998). Hellinger's transformations were performed on zooplankton biomass data (Legendre and Gallagher 2001). The significant explanatory variables (disturbances and environmental factors) were selected via forward selection and tested with a permutation Monte-Carlo test using 999 permutations; only variables with $P < 0.05$ were included in the model. Adjusted r^2 were calculated (Legendre and Legendre 1998). Variance partitioning analysis (VPA) was calculated from partial RDA analysis to assess the amount of variation explained independently by each set of explanatory factors, i.e., disturbances (Table 3), water quality (Table 2), and morphometry (Table 1). All RDAs were performed using CANOCO version 4.0 (ter Braak 1988, 1990).

Results

Residential development and land-cover disturbances within the watershed and the nearshore riparian strip (<50 m) are among the variables that accounted for 78.1% of the total variation among lakes (Fig. 2*a*). Along the first PCA axis explaining 59% of the total variation, human disturbances on the lakeshores (RD50, LC50) and within the watershed (RDW, LCW) were associated with enriched nutrients (TP, TDP, TN) and algal biomass (Chl *a*), decreased water transparency (Secchi), higher zooplanktivory indices (BLM_CL, BLM_D), shallower lakes, and flat watersheds. The second PCA axis explained 19.1% of the total variation among lakes; it was associated with decreases in watershed area, drainage, lake volume, and mean and maximum depths (Fig. 2*a*). Lakes Connelly and Truite (Fig. 2*b*, lower right side) had the highest level of residential development within the 50 m riparian strip, whereas Lakes Rond and Renée (Fig. 2*b*, upper right side) were the most disturbed within the entire watershed. Lakes Connelly and Truite, located on flat and large watersheds, had low relative depths and were less transparent. Lakes Rond and Renée showed the highest enrichment in nutrients and algal biomass. On the left side of the ordination plot (Fig. 2*b*), the most pristine lakes (Violon, Cabane, and Croche) were located on steeper watersheds and had more transparent waters and lower zooplanktivory, as indicated by the larger body length metrics of crustaceans, cladocerans, and *Daphnia*. The other lakes shared intermediate levels of disturbance and had variable environmental conditions.

Additionally, when looking at the linear relationships between disturbance variables and nutrients, the best positive relationships (higher r^2) were observed with the residential development disturbance (Table 4). Residential development within the watershed (RDW) was related to nutrient enrichment (TP and TN) but not to an increase in algal biomass (Chl *a*). Residential disturbance on large riparian strips (200–1000 m) was also associated with increases in TN concentrations, and nearshore residential disturbance (100–500 m) was associated with increases in littoral macrophyte biomass (Table 4).

Fig. 2. Ordination biplots of principal component analysis (PCA) for (*a*) the disturbance, lake and watershed morphometry, water quality, and planktivory variables and (*b*) the studied lakes. The most pristine lakes are encircled. See Tables 1, 2, and 3 for the codes of lakes and abbreviations of variable names.



Crustacean zooplankton communities

The relationship between mean summer crustacean density and residential development within the watershed was best described by a quadratic model ($r^2 = 0.828$, $P < 0.001$) as one datum (Lake Renée) strongly influenced the curvilinear relationship (Fig. 3*a*). Crustacean densities remained low until residential development reached a value close to 200 dwellings·km^{-2}. For mean summer crustacean biomass, a linear relationship with residential development was the best model ($r^2 = 0.628$, $P = 0.004$) (Fig. 3*b*). In addition, total crustacean biomass was significantly related to residential development within the watershed and the riparian zone >1000 m and to nutrient enrichment (TP, TN), but not to land-cover disturbance (Table 4).

Summer crustacean community structure varied among lakes showing dominance by *Daphnia* or by calanoids or

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Table 4. Regression analyses (r^2; coefficients of determination) between (*a*) residential development (RD) within the watershed and on different riparian zones and land-cover disturbance within the watershed (LCW) in the studied lakes ($n = 13$) versus summer mean (three months) of the water quality variables and biomass of total crustaceans and functional groups and (*b*) water quality variables and the biomass of total crustaceans and functional groups.

(*a*) RD and LCW vs. water quality variables and biomass of total crustaceans and functional groups.

	TP	TN	Macro	Crus	Bos	Cerio
RD100			+0.639 (0.002)			
RD150			+0.661 (0.001)			
RD200		+0.569 (0.003)	+0.662 (0.001)			
RD250		+0.605 (0.003)	+0.635 (0.002)			
RD500		+0.679 (0.001)	+0.527 (0.005)			
RD1000		+0.728 (<0.001)		+0.534 (0.005)		
RDW	+0.542 (0.005)	+0.668 (0.001)		+0.628 (0.002)		+0.546 (0.005)
LCW					+0.686 (0.001)	

(*b*) Water quality variables vs. biomass of total crustaceans and functional groups.

	Crus	Cerio	Dia	Cyclo
TP	+ 0.558 (0.005)			
TN	+ 0.540 (0.005)	+ 0.548 (0.005)	+ 0.521 (0.005)	+ 0.521 (0.005)

Note: Only significant relationships are shown with their probability in parentheses (Bonferroni correction: $P \leq 0.005$). TP, total phosphorus ($\mu g \cdot L^{-1}$); TN, total nitrogen ($\mu g \cdot L^{-1}$); Macro, macrophyte biomass ($mg \cdot L^{-1}$); Crus, total crustacean biomass ($\mu g \cdot L^{-1}$); Cerio, *Ceriodaphnia* biomass ($\mu g \cdot L^{-1}$); Dia, *Diaphanosoma* biomass ($\mu g \cdot L^{-1}$); Cyclo, cyclopoid biomass ($\mu g \cdot L^{-1}$). RD in riparian strips of increasing widths: 100 m, RD100; 150 m, RD150; 200 m, RD200; 250 m, RD250; 500 m, RD500; 1000 m, RD1000; RDW, entire watershed.

cyclopoids (Fig. 4*a*). In Lake Tracy, crustaceans were mostly composed of *Daphnia*, whereas calanoids were dominant in Lakes Violon and Morency, and cyclopoids were dominant in Lake Purvis. In Lake Nord, cyclopoids and calanoids were of equal importance. Although in low numbers, small cladocerans such as *Bosmina*, *Ceriodaphnia*, and *Diaphanosoma* were found more frequently in the most disturbed lakes (Rond and Renée). *Holopedium* was only found mainly in less disturbed lakes (Cabane, Croche, and Nord). Human disturbance within the watershed was related to increasing biomass of *Ceriodaphnia* (RDW) and *Bosmina* (LCW), and nutrient enrichment (TN) was associated with higher biomass of *Ceriodaphnia*, *Diaphanosoma*, and cyclopoids (Table 4). In contrast, changes among lakes in the biomass of calanoids, *Daphnia*, and *Holopedium* were not related to human disturbance or nutrient enrichment.

Daphnia assemblages also varied greatly among lakes. Two small species (*D. ambigua* and *D. g. mendotae*) were dominant in Lake Renée, and *D. g. mendotae* was the sole species present in Lakes Rond, Connelly, and Morency (Fig. 4*b*). Another small species (*D. dubia*) was found in low numbers in two lakes (Purvis and Truite). The large species *D. pulicaria* was abundant in three lakes (Tracy, Truite, and Blanche), and *D. catawba* was abundant in three others (Gervais, Croche, Violon). Finally, *D. longiremis* was found in high numbers in three lakes (Croche, Cabane, and Purvis) and in low numbers in three others (Nord, Blanche, and Truite) (Fig. 4*b*).

LCC cascades

All LCC models began with residential development within the watershed (RDW) and land-cover clearing within the watershed (LCW in two cases only) as the main disturbance stimulus, and they included one of the potential intermediate variables as the linking element (Figs. 5 and 6). RDW was the strongest disturbance stimulus retained in most LCC models, and TP concentrations was the most common intermediate element.

LCC models explained between 40% and 73% of among-lake variation in the biomass of crustacean functional groups (Fig. 5). The strongest cascades were observed for the small cladocerans. RDW increased the biomass of small cladocerans (*Bosmina*, *Ceriodaphnia*, and *Diaphanosoma*) through TP enrichment. RDW also increased the biomass of cyclopoids through intermediate algal enrichment (Chl *a*). Cascading effects of RDW on *Daphnia*, calanoids, and *Holopedium* were weak and mediated by changes in the depth of the euphotic zone. In addition, RDW was associated with decreasing cladoceran or *Daphnia* body length metrics, indicating higher zooplanktivory in the most developed lakes. The biomass of *Ceriodaphnia*, *Diaphanosoma*, and calanoids increased with lower zooplanktivory, as indicated by the negative correlations with *Daphnia* body length metrics. Only *Daphnia* showed an independent relationship with lake depth; *Daphnia* biomass decreased in deeper lakes.

LCC models explained between 38% and 74% of among-lake variation in the biomass of *Daphnia* species (Fig. 6). Through TP enrichment, RDW was related to increasing biomass of small *Daphnia* species (*D. g. mendotae*, *D. dubia*, and *D. ambigua*). In contrast, cascading effects of RDW on the biomass of *D. longiremis* and large species (*D. pulicaria* and *D. catawba*), through TP enrichment, were weak. The LCC models for *D. pulicaria* and *D. catawba* revealed instead a strong independent effect of low zooplanktivory, as indicated by high positive correlations between cladoceran body length metrics (BLM_CL) and the biomass of large *Daphnia* species. For the other *Daphnia* species, correlations with this zooplanktivory index were weak. Chl *a* was a significant intermediate element only in the *D. dubia* model.

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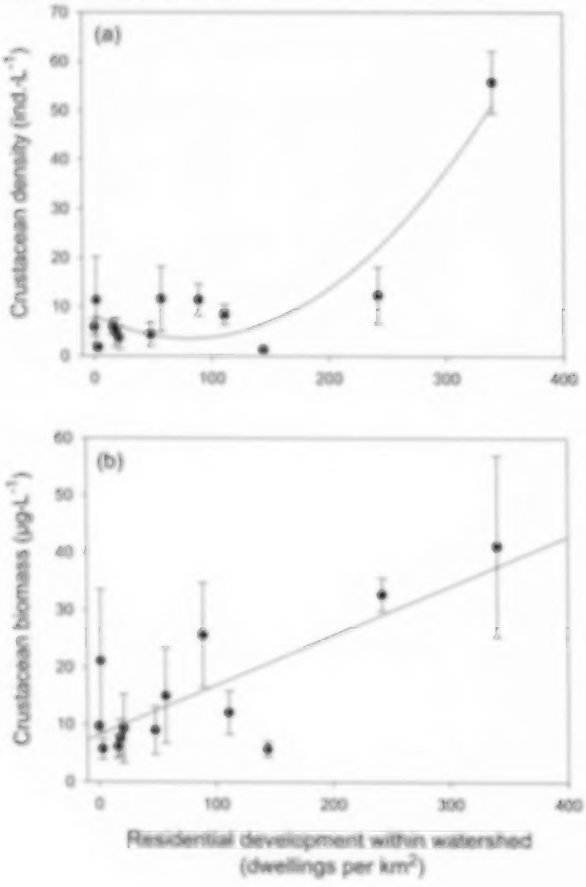

Fig. 3. Relationships between (*a*) mean summer density (individuals (ind.)·L^{-1}) and (*b*) mean summer biomass (µg dry weight·L^{-1}) of total crustacean zooplankton and the level of residential development within the watershed (number of dwellings·km^{-2}). Error bars are the standard errors (n = 3).

Relative influence of disturbance and environmental factors

RDW, TP enrichment, and zooplanktivory indices explained 42.2% of among-lake variation in crustacean community structure (P = 0.001) (Fig. 7*a*). The four significant variables were the zooplanktivory indices (BLM_CL, 22%, P = 0.001; BLM_D, 11%, P = 0.001), RDW (13%, P = 0.003), and total phosphorus enrichment (TP, 5%, P = 0.016). Along axis 1, the gradient of residential development was associated with higher biomass of two small cladocerans (*Diaphanosoma* and *Ceriodaphnia*) and lower biomass of calanoids, whereas *Bosmina* had an intermediate position. Along axis 2, TP enrichment was associated with higher biomass of cyclopoids and lower biomass of *Daphnia*. Higher *Daphnia* biomass was also strongly related to low zooplanktivory, as indicated by increasing size of cladocerans and *Daphnia* in the less TP enriched lakes. Variance partitioning analysis indicated that the two zooplanktivory indices explained most of the variation in crustacean community structure (26%), whereas residential development and TP enrichment explained together 12% of the variation. Around 7% of the variance was explained by interactions among disturbance, nutrient, and zooplanktivory variables.

RDW, zooplanktivory indices, lake depth, and macrophyte biomass together explained 57.3% of among-lake variation in *Daphnia* species assemblages (P = 0.001) (Fig. 7*b*). Zooplanktivory indices again made the greatest contributions (BLM_D, 27%, P = 0.001; BLM_CL, 12%, P = 0.002), followed by macrophyte biomass (Macro, 9%, P = 0.005), residential development within the 50 m riparian strip (RD50, 8%, P = 0.001) and within the watershed (RDW, 4%, P = 0.049), and lake maximum depth (Z_{max}, 1%, P = 0.02). Along axis 1, large *Daphnia* species (*D. catawba* and *D. pulicaria*) were associated with low zooplanktivory in deep lakes and were negatively related to the small species *D. g. mendotae*, which was associated with higher biomass of littoral macrophytes and important residential development (Fig. 7*b*). *Daphnia longiremis* was negatively associated with the residential development gradient. Again, variance partitioning analysis indicated that zooplanktivory indices explained 23.9% of among-lake variation in *Daphnia* species assemblages, whereas residential disturbance accounted for 11.3% and lake depth and macrophyte biomass accounted for 15.9%. Approximately 6% of variation in *Daphnia* species was explained by interactions among variables.

Discussion

Our study provides partial support to the hypothesized cascading effect of recent watershed urbanization on crustacean zooplankton in nutrient-poor boreal lakes. Zooplanktivory is also an important driver of changes in crustacean zooplankton community. In fact, land-use modifications resulted in both bottom-up effects through nutrient enrichment and top-down effects via angling pressure on piscivores, as suggested by the influence of zooplanktivory indices. The major disturbance stimulus was the level of residential development and its association with increased TP, TN, and crustacean abundance, even though the majority of lakes were still in an oligotrophic state. Residential development had a greater influence on TP enrichment and crustacean community than did the clearing of forested lands. The recent transition of pristine lacustrine environments to urbanized and recreational lands increased the flow of nutrients into aquatic systems and indirectly affected crustacean zooplankton community in boreal lakes, as seen in other north-temperate lakes and reservoirs (Knoll et al. 2003; Hoffmann and Dodson 2005).

Large-scale residential development within the watershed rather than small-scale riparian disturbance was the major disturbance stimulus related to responses of pelagic crustacean zooplankton. This finding runs counter to the responses of littoral communities of macrophytes, periphyton (Lambert et al. 2008), and benthic macroinvertebrates (De Sousa et al. 2008), which responded to nearshore residential development and land-cover disturbances in the same lakes. Schindler et al. (2000) also found that fish growth was negatively affected by residential development of lake shores in north-

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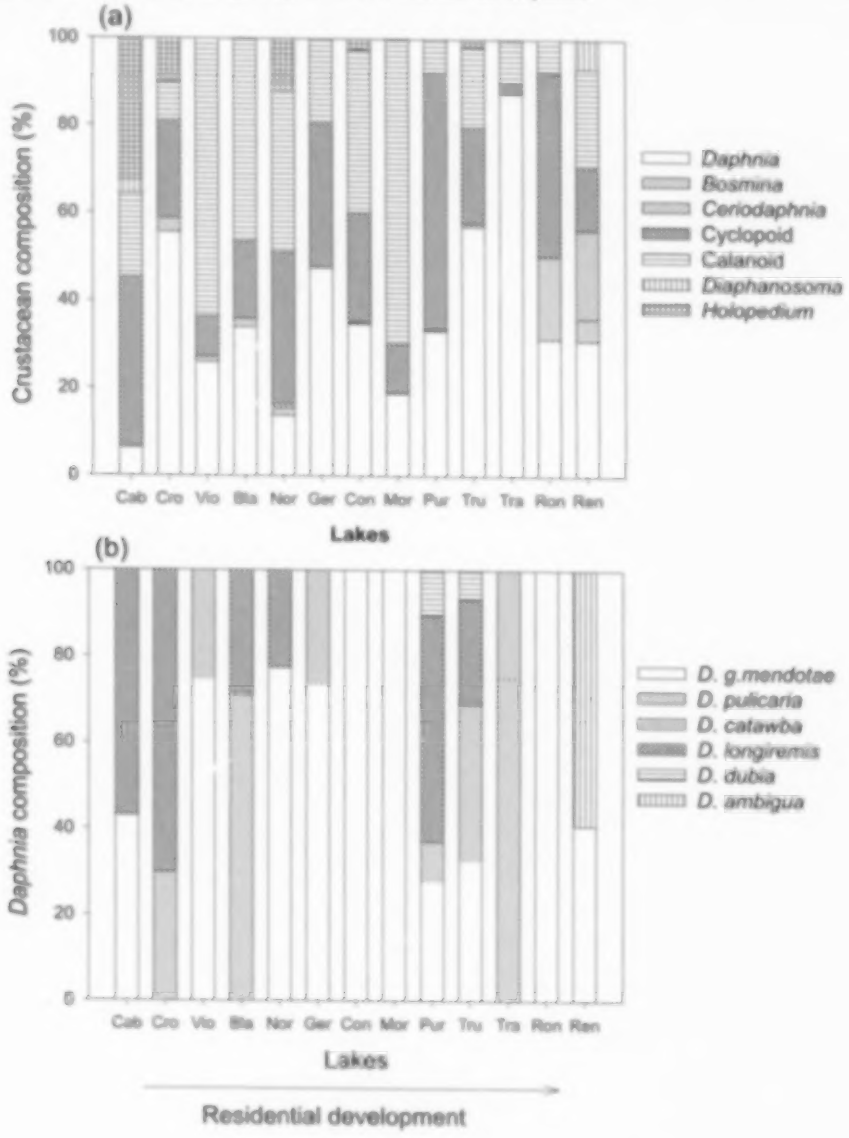
Fig. 4. Relative abundance (as percentage of mean summer biomass) of (*a*) crustacean functional groups (*Bosmina*, *Holopedium*, *Daphnia*, *Diaphanosoma*, *Ceriodaphnia*, calanoid, and cyclopoid) and (*b*) *Daphnia* spp. (*D. pulicaria*, *D. catawba*, *D. longiremis*, *D. g. mendotae*, *D. dubia*, and *D. ambigua*) in the 13 lakes along the gradient of residential development.



temperate lakes. Human land use increases lake productivity by promoting nutrient transfers from watersheds to lakes (Lamontagne et al. 2000; Knoll et al. 2003; Hoffmann and Dodson 2005), likely affecting the littoral area before the pelagic zone. Our study suggests that large-scale residential disturbance within the watershed can indirectly increase crustacean density and biomass in the pelagic zone, at least when residential development reaches more than 200 dwellings·km^{-2}. Further, such disturbances of nearshore residential development can have an early impact on littoral communities (De Sousa et al. 2008; Lambert et al. 2008).

In LCC models, TP enrichment was the best intermediate element linking residential disturbance to changes in crustacean zooplankton biomass, especially for the small cladocerans. Our finding provides additional support to the hypothesis that TP is the best predictor of changes in crustacean zooplankton biomass in the Laurentian lakes, as in other Canadian nutrient-poor lakes (Yan 1986), and along latitudinal and trophic gradients (Pinto-Coelho et al. 2005). Crustacean community shifted towards functional groups of smaller body sizes in the more developed lakes. Thus, for *Bosmina*, *Ceriodaphnia*, *Diaphanosoma*, and small *Daphnia*

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Fig. 5. Path diagrams of land-cover cascade (LCC) models quantifying cascades between watershed disturbances and the biomass of each crustacean functional group. Ovals indicate human disturbance stimuli (RDW, LCW) or the responses of crustacean functional groups, and rectangles indicate intermediate elements. Total variation explained by the LCC model for each crustacean functional group is indicated at the bottom; path coefficients for intermediate elements are located below each significant variable. Abbreviations: TP, total phosphorus; Chla, chlorophyll *a*; EZ, euphotic zone depth; Macro, macrophyte biomass; BLM_CL, cladoceran body length metric; BLM_D, *Daphnia* body length metric.

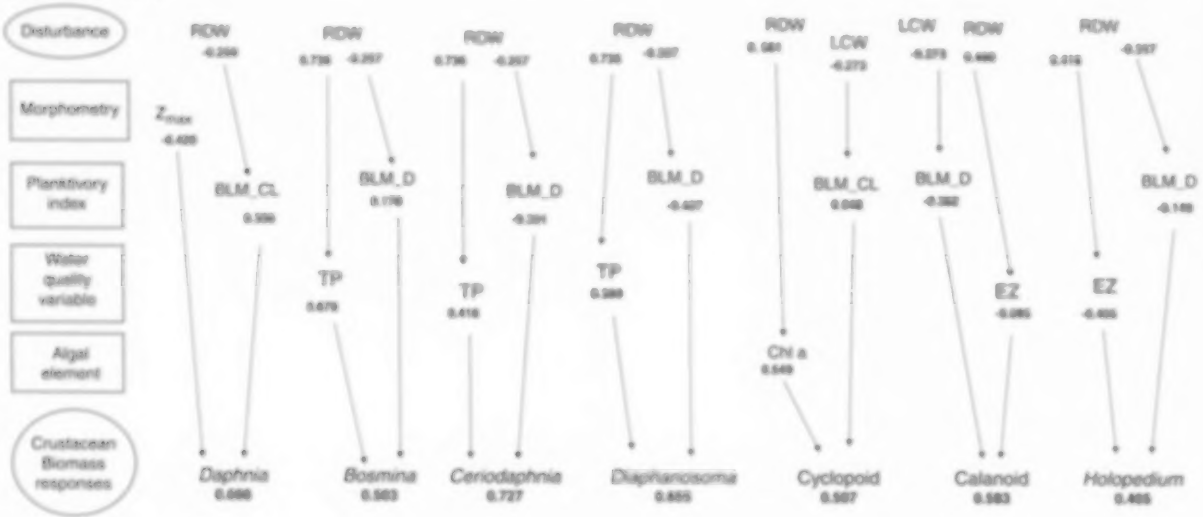
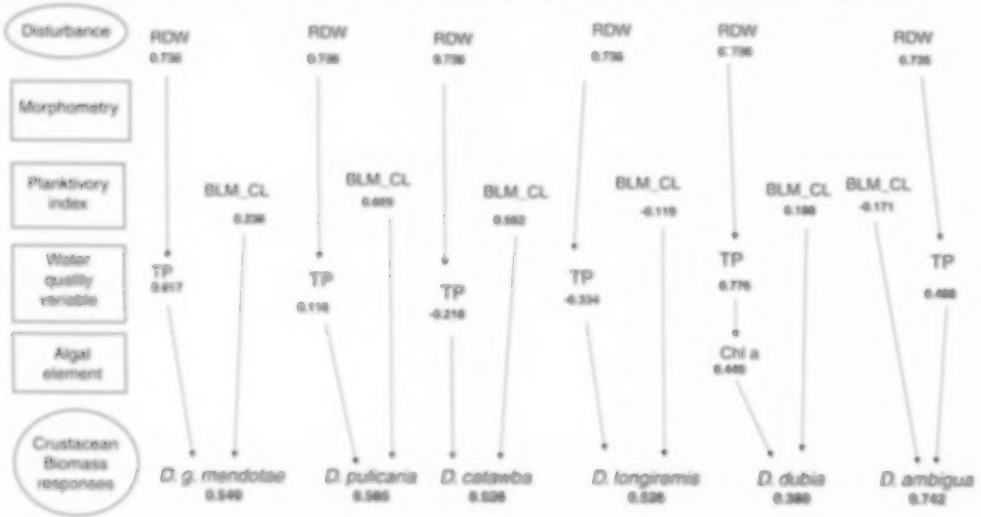


Fig. 6. Path diagrams of land-cover cascade (LCC) models quantifying cascades between watershed disturbances and the biomass of each *Daphnia* species. Ovals indicate human disturbance stimuli (RDW, LCW) or the responses of *Daphnia* species, and rectangles indicate intermediate elements. Total variation explained by the LCC model for each *Daphnia* species is indicated at the bottom; path coefficients for intermediate elements are located below each significant variable. Abbreviations: TP, total phosphorus; Chla, chlorophyll *a*; EZ, euphotic zone depth; Macro, macrophyte biomass; BLM_CL, cladoceran body length metric; BLM_D, *Daphnia* body length metric.



species (*D. g. mendotae*, *D. dubia*, and *D. ambigua*), both the LCC models and redundancy analysis showed a stronger response of these species to TP enrichment and a weaker response to the zooplanktivory index. In addition, negative correlations with *Daphnia* body length for *Diaphanosoma* and *Ceriodaphnia* could suggest competitive interactions with large *Daphnia*. Indeed, *Diaphanosoma* is mostly found in meso-eutrophic lakes in the absence of *Daphnia* (Stich 2004).

Other environmental factors such as phytoplankton biomass and the depth of the euphotic zone were linking elements in LCC models of cyclopoids and *Holopedium*. These

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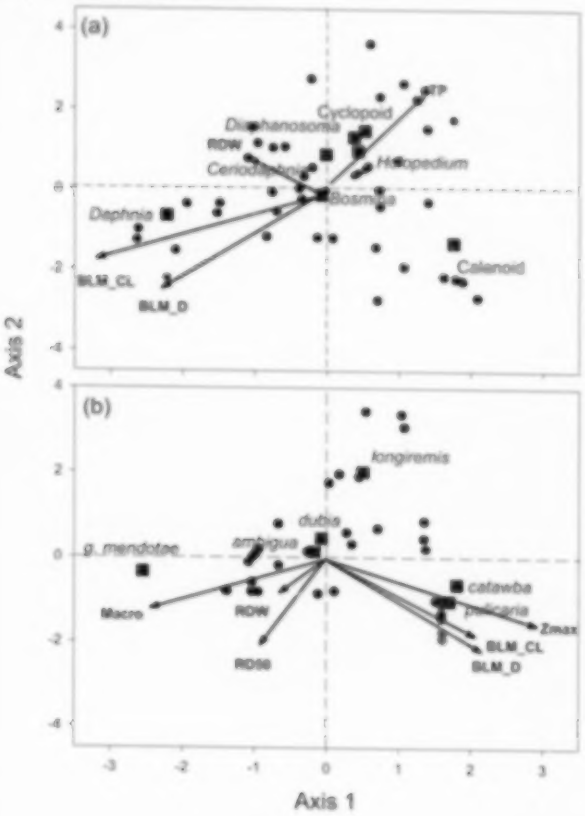
Fig. 7. Ordination biplots of redundancy analyses (RDAs) for the analysis of the relationships between watershed disturbance, TP enrichment, planktivory, lake morphometry, and macrophytes and (*a*) the crustacean community structure based on the relative abundance (biomass) of crustacean functional groups and (*b*) *Daphnia* species assemblages. The small circles represent lakes and the large squares represent (*a*) the crustacean functional groups or (*b*) *Daphnia* species. TP, total phosphorus; RDW, residential development within the watershed; RD50, residential development within the 50 m riparian strip; Z_{max}, maximum depth; Macro, macrophyte biomass; BLM_CL, cladoceran body length metric; BLM_D, *Daphnia* body length metric.



species may respond to subtle indirect effects of residential development on algal community and water transparency, even though no correlations were found between residential development and Chl *a* or Secchi transparency. Patoine et al. (2000) also found that calanoid copepods were sensitive to intensive disturbance of watersheds by clearcut logging. The longer development time of copepods likely makes them more sensitive to disturbance and less able to flourish in affected environments.

Daphnids were negatively associated with TP but were also found in some of the most developed lakes. This could reflect the potential of *Daphnia* as an effective filter feeder capable of creating a clear-water phase through their high grazing efficiency and thus reducing algal biomass and TP (Lathrop et al. 1999; Beisner 2001; Stemberger and Miller 2003). However, neither Secchi water transparency nor the depth of the euphotic zone was significantly related to *Daphnia* biomass in our study. In fact, *Daphnia* was more strongly related to low zooplanktivory than to residential development, as indicated by the positive correlations between *Daphnia* biomass and the zooplanktivory indices in the LCC models. Furthermore, the zooplanktivory indices and the degree of residential development within the watershed were two independent gradients.

In the LCC models, daphnids were the sole crustacean group associated with changes in morphometry of lakes, which were independent of the level of residential disturbance. Daphnid biomass was negatively correlated with maximum lake depth, but no individual species were. This was a surprising result, given that we expected a positive correlation between maximum depth and *Daphnia* biomass because of potential vertical migration and use of deeper strata by *Daphnia* as a refuge from predation (Lampert 1989; Wright and Shapiro 1990). This result should be interpreted with caution because *Daphnia*, as a functional group, included all species of different sizes, and small *Daphnia* species were found to be more abundant in shallow and developed lakes. Nonetheless, results from the redundancy analysis showed that the large-bodied *D. pulicaria* and *D. catawba* were found in greater abundance in deep lakes that had low zooplanktivory but were not related to disturbance by residential development. These large *Daphnia* are generally found in deep lakes (Keller and Conlon 1994) or reside in deeper zones (Gélinas and Pinel-Alloul 2008) where they can avoid predation. Large *Daphnia* undergo diurnal vertical migration to reduce mortality by moving to deeper water where darkness provides a visual refuge from fish (Lampert 1989; Wright and Shapiro 1990). In contrast, higher abundance of the small-bodied *D. g. mendotae* in the most developed, nutrient-enriched, and high zooplanktivory lakes indicated that this small species was better able to avoid predation owing to inducible morphological and behavioural defenses (Gélinas et al. 2007) and the presence of refuge in littoral macrophytes (Burks et al. 2002). However, the small hypolimnetic species *D. longiremis* declined in abundance with increasing residential development.

Dodson et al. (2005) pointed out that modern zooplankton ecologists are faced with understanding the relative impacts of anthropogenic and natural ecological factors on pelagic communities. Our study showed that crustacean zooplankton community and *Daphnia* species assemblages varied with the degree of residential disturbance of lake watersheds, while also responding to other environmental factors associated with predation pressure. It is still difficult to separate the effects of interactions among residential development, nutrient enrichment, and zooplanktivory because of among-lake differences in cottaging, recreational activities, and sport fishing. VPA demonstrated that the zooplanktivory index was more important than residential development and nutrient enrichment in explaining among-lake variation in crustacean community structure. We also found a stronger structuring effect of zooplanktivory than of residential development on *Daphnia* species assemblages. Predation pressure appeared to exert an independent effect on *Daphnia* species larger than 2 mm (*D. pulicaria* and *D. catawba*). The presence of refuges in deep lakes and littoral macrophytes also influenced *Daphnia* species assemblages.

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Other unmeasured environmental factors also likely affected crustacean zooplankton community structure in the study lakes. Specifically, our analyses captured only 42% of the total variation in the crustacean community and 58% in *Daphnia* assemblages. Therefore, more factors and their potential interactions could have had both direct and complex nonadditive impacts on the plankton of these and other nutrient-poor boreal lakes (e.g., Christensen et al. 2006). The observed effects on zooplankton community structure may have been attributable to among-lake differences in colonization events, unmeasured abiotic and biotic variables, and temporal variation in urbanization and land use over the past 30 years. Unfortunately, the exact dates of the major land-use changes for a given lake are unknown. Similar alterations in abundance, body size, species richness, and species composition were observed in a long-term study in Dorset lakes (Yan et al. 2008). For example, rapid zooplankton species turnover rate (23%) occurred in Lake Heney over a period of 12 years as a result of recovery from acidification and introduction of the zooplankton invader *Bythotrephes* (Arnott et al. 1999). Although the study lakes in the Laurentian area have not been stressed by acidification and exotic zooplankton, these relatively pristine lakes have experienced stressors associated with three decades of cultural eutrophication.

Eutrophication by residential development of watersheds has become one of the most pervasive problems in recreational lakes, and limnologists should develop biomonitoring tools to assess the status of these ecosystems. Recent studies showed that residential development within the watershed might subtly affect littoral (De Sousa et al. 2008; Lambert et al. 2008) and pelagic (this study) communities even in relatively pristine Canadian Shield lakes that are protected from more intense disturbances by agriculture and industrial activity. In comparison, certain extreme events of eutrophication such as anoxia at night, high pH during the day, and blooms of cyanobacteria are still relatively infrequent in these lakes.

Crustacean zooplankton are a key element of the pelagic food web (McQueen et al. 1986; Carpenter and Kitchell 1993), and thus, any change in the community structure, particularly among cladocerans and *Daphnia* species of different body sizes, will have profound functional impacts. The identification of well-defined functional groups may help to establish biomonitoring programs based on efficient bioindicators (Stemberger et al. 2001). Crustacean zooplankton assemblages are complex and contain many groups and species that are capable of responding differently, and in unpredictable ways, to watershed disturbance (Stemberger and Lazorchak 1994; Patoine et al. 2000; Dodson et al. 2005). Our study indicated that some crustacean functional groups and *Daphnia* species could function as bioindicators of watershed disturbance in the Laurentian lakes that have recently begun undergoing residential development. Small cladocerans (*Bosmina*, *Ceriodaphnia*, and *Diaphanosoma*) and *Daphnia* (*D. g. mendotae*) were indicative of the cascading impact of residential development through TP enrichment and increasing zooplanktivory. Sensitive calanoid copepods and the large *Daphnia* (*D. pulicaria* and *D. catawba*) responded the most to the disturbance and zooplanktivory gradients, and their presence reflects more pristine conditions in deep lakes. Our study provides potential biomonitoring tools based on crustacean zooplankton community structure to assess the response of pelagic communities to watershed disturbance at large-scale levels, while two companion studies on littoral communities (De Sousa et al. 2008; Lambert et al. 2008) have offered complementary tools based on periphyton and macroinvertebrate communities.

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Precipitous declines in pinto abalone (*Haliotis kamtschatkana kamtschatkana*) abundance in the San Juan Archipelago, Washington, USA, despite statewide fishery closure

Don P. Rothaus, Brent Vadopalas, and Carolyn S. Friedman

Abstract: Pinto abalone (*Haliotis kamtschatkana kamtschatkana*) index stations in the San Juan Archipelago were systematically monitored by the Washington Department of Fish and Wildlife from 1992 through 2006. During this period, abalone abundance declined by 77% and the mean shell length (SL) increased 10.4 mm. Abalone densities at all index stations are currently well below the threshold of 0.15 abalone·m^{-2} required for successful fertilization. From 1992 to 1996, 16% of individuals encountered measured <90 mm SL, while only 6% of the individuals from 2003 to 2006 were in this small size class. Similarly, the number of those >114 mm SL was greater in the 2000s than in the 1990s. The mean SL of all live abalone observed in the 1990s (107.62 ± 0.87 mm) was significantly different from the mean SL of empty shells (114.21 ± 2.1 mm), but no difference was detected between the mean SLs of empty shells and live abalone in the 2000s (114.97 ± 1.42 mm). Taken together, these data suggest recruitment failure from an Allee response to low population densities.

Résumé : Le ministère de la pêche et de la faune de l'état de Washington (Washington Department of Fish and Wildlife) a suivi systématiquement les stations de référence de l'ormeau nordique (*Haliothis kamtschatkana kamtschatkana*) dans l'archipel de San Juan de 1992 à la fin de 2006. Durant cette période, l'abondance des ormeaux a diminué de 77 % et la longueur moyenne des coquilles (SL) a augmenté de 10,4 mm. Les densités des ormeaux à toutes les stations de référence sont actuellement bien en deçà du seuil de 0,15 ormeau·m^{-2} requis pour une fécondation réussie. En 1992–1996, 16 % des individus observés avaient une SL < 90 mm, alors qu'en 2003–2006 seuls 6% des individus appartenaient à cette classe de petite taille. De même, le nombre d'individus avec une SL > 114 mm était plus important durant les années 2000 que durant les années 1990. La longueur moyenne des coquilles de tous les ormeaux vivants observés durant les années 1990 (107,62 ± 0,87 mm) est significativement différente de la longueur moyenne des coquilles vides (114,21 ± 2,1 mm), mais il n'y a pas de différence détectable entre les longueurs moyennes entre les coquilles vides et celles des ormeaux vivants durant les années 2000 (114,97 ± 1,42 mm). Dans leur ensemble, ces données laissent croire à un échec du recrutement dû à une réaction de type Allee aux faibles densités de population.

[Traduit par la Rédaction]

Introduction

Successful fishery management requires the ability to predict sustainable harvest quotas, which is often difficult for species with periodic spawning events, varying dispersal, and recruitment (Hobday et al. 2001). Overexploitation of broadcast spawning marine invertebrates, such as abalone, is often due to a paucity of information on life history characteristics and population dynamics coupled with the incorrect assumption that the combination of high fecundity, broadcast spawning, and planktonic larvae buffer against fishing impacts (Hobday et al. 2001).

Abalone populations may be more highly prone to extirpation than many exploited species for several key reasons: (*i*) they are sedentary and contagiously distributed, giving rise to serial depletion of beds (Karpov et al. 2000; Hobday et al. 2001); (*ii*) they typically occur in shallow subtidal areas and may have no deepwater refuge from harvesters; (*iii*) abalone are exceptionally prized by fishers and consumers; (*iv*) recruitment may be dependent on localized larval production and settlement owing to their 5- to 10-day planktonic larval stage (e.g., Prince et al. 1987; Chambers et al. 2006; Miner et al. 2006); (*v*) the cryptic nature of juveniles combined with the longevity and slow growth of adults may mask recruitment failure over several years, complicating fishery management of abalone (Rogers-Bennett et al. 2004); and (*vi*) long-lived, broadcast spawning invertebrates, like abalone, may exhibit high interannual recruitment variability (Hobday et al. 2001).

Given this vulnerability to fishing, it is not surprising that many abalone fisheries worldwide have collapsed (Sloan and Breen 1988; Tegner 1993; Shepherd et al. 2001). Pinto

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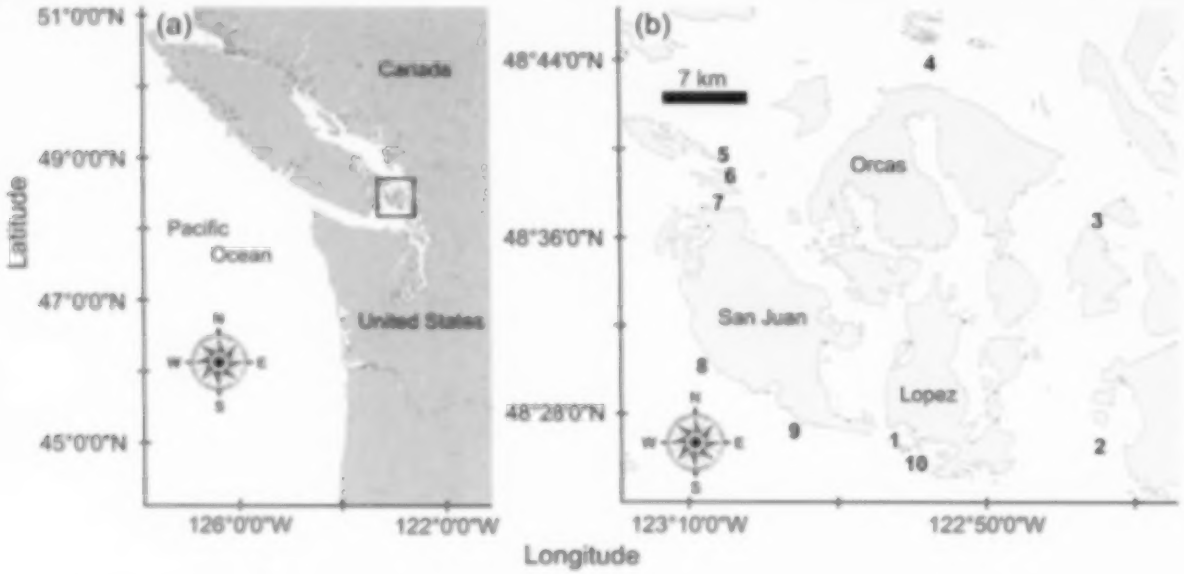
Fig. 1. General area map (*a*) and vicinity map (*b*) of *Haliotis kamtschatkana* index stations in the San Juan Archipelago, Washington State, USA.



abalone (*Haliotis kamtschatkana*), inhabiting the eastern Pacific rim of North America, are no exception and have declined throughout their range (Gesselbracht 1991; Campbell 2000; Woodby et al. 2000). Pinto abalone are dioecious broadcast spawning mollusks. Although age estimation has not been possible using shell characteristics (Fournier and Breen 1983), mark–recapture studies suggest a maximum age of 50 years (Breen 1980*a*). The 5- to 10-day lecithotrophic pelagic larval period, which likely limits dispersal (Tegner 1993), concludes with torsion and metamorphosis to the adult form. Juveniles are cryptic, first feeding on benthic diatoms and, as size increases, add seasonally available benthic macroalgae to their diet. Pinto abalone mature and become emergent at approximately 50 mm shell length (SL) (Quayle 1971; Paul and Paul 1981; Boutillier et al. 1985). Because low densities can negatively affect fertilization success in broadcast spawning marine invertebrates (Allee et al. 1949), declines in pinto abalone abundance can result in population collapse, even in the absence of continued fishing (e.g., Hobday et al. 2001).

In British Columbia, Canada, small-scale commercial harvest of pinto abalone began early in the 20th century (Sloan and Breen 1988); large-scale commercial dive fishing began in 1952, with the largest landings occurring in 1978. This dive fishery lasted 38 years but was closed, along with the recreational fishery, in 1990 as a result of population declines (Winther et al. 1995). Because of sustainability concerns, a commercial pinto abalone fishery was never permitted in Washington; unfortunately, annual harvest records were not collected for the recreational fishery. The Washington Department of Fish and Wildlife (WDFW) closed the recreational fishery in 1994 owing to abundance declines. Prior to the closure, annual harvest estimates ranged from 38 200 to 40 934 abalone (Bargmann 1984 and Gesselbracht 1991, respectively). As a result of these regional declines in abundance, the pinto abalone was listed as a threatened species in 1999 by the Canadian government (COSEWIC 1999), a state candidate species in 1998 by WDFW, and a federal species of concern by the American NOAA (NOAA–Fisheries 2004).

Estimating the total abundance of abalone is not practical with direct stock surveys because of their highly contagious distribution, their cryptic nature, and their association with microhabitats (Sloan and Breen 1988). However, changes in abalone abundance can be detected at repeatedly surveyed sites (Breen 1980*b*). In this study, we analyze the spatial and temporal changes in abundance, density, size, and depth distribution of abalone in the San Juan Archipelago (SJA). In particular, we ask whether a relationship exists among the rate of decline and initial density, proximity to major freshwater inputs, or depth. We also ask whether there has been a substantial shift in length frequencies that may be indicative of pinto abalone recruitment trends in the SJA.

Materials and methods

Index stations

Ten sites around the SJA were chosen for long-term monitoring (Fig. 1). No attempt was made to randomly select sites; instead, sites were chosen that were distributed around the SJA and were known from dive surveys to contain abalone at densities that would likely result in reproduction and also attract divers. Such sites tend to be composed mostly of bedrock, boulders, or some combination of the two (Table 1). This habitat is generally encrusted with crustose corraline algae (CCA) with abundant macroalgae and kelp assemblages (D. Rothaus, personal observation). Other factors used in choosing the sites were their proximity to visual landmarks (for visual alignment and laser range-finding fixes) and local current patterns (areas subject to

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Table 1. Characteristics of *Haliotis kamtschatkana* index stations established in 1992 in the San Juan Island Archipelago, Washington State.

Station No.	Name	Area (m^2)	Shallow (%)	Deep (%)	Site description
1	Long Island West	152	50	50	Flat bedrock plateau with small boulders and steep slope at deep boundary and west side
2	Williamson Rocks	190	80	20	Angled bedrock with numerous small and large boulders; shoreline point creates wedge-shaped wall on nearshore boundary
3	North Cypress Island	316	48	52	Large and small boulders on gravel bottom with occasional hardpan
4	Parker Reef	375	15	85	Flat bedrock with a few cracks, crevices, two–three large boulders, small patch of cobble, and small boulders on northwest boundary
5	Ripple Island	135	90	10	Two flat bedrock plateaus separated by a diagonal undercut wall, cobble and small boulders on upper plateau
6	Big Cactus Island	158	75	25	Bedrock, steeply sloping at shallow boundary to flat plateau with cobble and small boulders, steeply sloping again at deep boundary
7	Spieden Island	176	80	20	Smooth, steeply sloping bedrock wall with two or three diagonal cracks
8	Deadman's Bay	155	65	35	Bedrock wall with longshore cracks, a few boulders at north and south end
9	Old Eagle Point	229	40	60	Bedrock, steeply sloping at shallow boundary to flat plateau with cobble and small boulders
10	Long Island East	356	35	65	North boundary steeply sloping bedrock with downslope crevices, changing to medium boulders along south boundary
Average		224	58	42	

extreme currents could not be reliably surveyed). During the initial surveys (1992), the sampling occurred in November. Because of the high density of remnant macroalgae during this time of year, subsequent surveys (1994–2006) were conducted during February and early March when macroalgae is typically at its lowest density.

Sites ranged in area from 135 to 375 m^2, with a mean of 224 m^2 (Table 1). Each site is a roughly rectangular polygon with its long dimension parallel to the shoreline; each corner was permanently marked by pitons flagged with a length of yellow polypropylene line to aid in relocation. Latitude–longitude information was acquired for each index site using differential global positioning system (GPS) equipment. Additionally, at each site distances from landmarks along the shoreline were triangulated using laser range equipment. Average depth of the sites was 4.5 ± 0.5 m confidence interval (CI) (all depth data corrected to chart datum, mean lower low water). Depth ranged by site, with shallow depth boundaries from 0.5 to 4.2 m and deep boundaries from 4.3 to 9.0 m. Prior to each survey, metre-delineated survey tapes were stretched between pitons to completely outline the site boundaries. Every site was fully censused during each survey after being subdivided into vertical sectors approximately 3 m in width. Divers recorded the location, size (maximum SL in mm), and depth (m) of each abalone encountered starting at the deep boundary and moving up to the shallow boundary of each sector. All empty shells (mortalities) encountered within sites were also measured and recorded. We separated shallow and deep strata at the 4.3 m depth contour and used habitat maps created for this study to calculate strata areas for each index site (Table 1).

Validation of survey method

The potential for positive and (or) negative bias (e.g., from contagious distribution and site-specific extirpation, respectively) exists in our fixed site survey strategy. Therefore, to investigate whether data from our index site surveys were reasonable representations of abalone densities at each locale, we collected additional density data at Long Island (near index plots Nos. 1 and 10) using a random–systematic survey method. We surveyed eleven 1 m wide transects spaced approximately 300 m apart that encompassed depths between 8 and 0 m. Divers recorded the dominant substrate type, the number and size of pinto abalone, and associated macrofauna for each 1 m^2 quadrat along each transect; numbers of quadrats per transect varied (n = 14 to 107) with depth gradients. Of the total 478 m^2 surveyed, 70% was considered suitable habitat for abalone based on the presence of CCA, bedrock, boulders, and (or) rugose substrate. We used these data to derive additional estimates of abalone density and mean SL at this locale for comparison with index site estimates.

Statistical analysis

Abundance change over time at depth was tested by analysis of covariance (ANCOVA) with negative binomial errors (log link). Density change over time as a function of initial density, proximity to the Fraser–Skagit rivers, and depth was tested by ANCOVA (normal errors). Density was tested as a

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function of site, initial density, depth strata area (mostly deep sites (4, 9, and 10); mostly shallow sites (2, 5, 6, 7, and 8); and balanced sites (1 and 3); see Table 1 and Fig. 1), and proximity to the Fraser and Skagit rivers (high–low) by ANCOVA. Model simplification was conducted by penalized log likelihood. Differences in density estimates and mean SL between the index site and transect methods were tested with Student's *t* tests. Differences in mean SL between mortalities and live specimens among sites and years were tested by analysis of variance (ANOVA). To test for differences in the proportion of (*i*) live individuals with SL < 90 mm (emergent recruits) and (*ii*) live individuals with SL > 114 mm (114 mm being the mean SL of mortalities), we initially used a general linear model with binomial errors; decadal differences were tested using a two-sample test for equality of proportions with continuity correction. These tests were conducted as implemented by S-Plus (Insightful Corporation, Seattle, Washington).

Results

Abundance

Overall average abundance in the SJA declined from 1992 to 2006 (*t* test, $t = 7.527$, df = 4, $p = 0.0017$). In the 1990s, abundance in the deep stratum was significantly higher than that in the shallow stratum ($t = 3.1256$, df = 4, $p = 0.0353$). In the 2000s, shallow abundance was higher than deep, but the difference was not significant ($t = 2.395$, df = 4, $p = 0.0748$). The decline in abundance of pinto abalone in the deep stratum was significantly faster than that for the shallow stratum (ANCOVA, $F = 17.8805$, df = 1, $p = 0.0029$, Fig. 2).

Density

Overall, average density declined from 0.18 to 0.04 abalone·m^{-2} from 1992 to 2006. Some sites experienced significantly greater rates of decline than others (ANCOVA, $F = 4.0441$, df = 9, $p = 0.0009$, Fig. 3). A positive relationship was detected between high initial density and decline in density (ANCOVA, $F = 4.336$, df = 1, $p = 0.0419$); a positive relationship was also detected between declines in density and sites with a high proportion of deep stratum (ANCOVA, $F = 6.721$, df = 1, $p = 0.0121$). No significant relationship was detected between site proximity to freshwater input and declines in density (ANCOVA, $F = 0.9588$, df = 1, $p = 0.3317$).

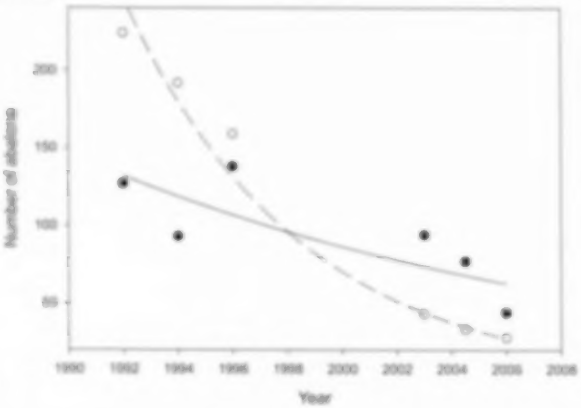
Survey methods

No differences were detected between density estimates (*t* test, $t = 1.8477$, df = 11, $p = 0.0917$) or mean SLs ($t = 0.3657$, df = 99, $p = 0.3577$) derived from the index site versus the random–systematic transect methods.

Size

No within-decade differences in overall mean SL (1990s: ANOVA, $F = 1.9211$, df = 2, $p = 0.1471$; 2000s: ANOVA, $F = 0.2201$, df = 2, $p = 0.8026$), the proportion < 90 mm, or the proportion > 114 mm were detected (1990s: $\chi^2 = 2.1800$, df = 2, $p = 0.3362$; 2000s: $\chi^2 = 0.4514$, df = 2, $p = 0.7980$). In the 1990s, mean SL in the deep stratum (107.1–110.9 mm, 95% CI) was significantly greater than that in the shallow stratum (101.7–104.5 mm, 95% CI; ANOVA, $F = 25.4138$, df = 1, $p < 0.0001$, Fig. 4). In the 2000s, no difference in mean SL was detected between depth strata (shallow 112.6–116.4 mm, deep 112.5–118.3 mm, 95% CI; ANOVA, $F = 0.47059$, df = 1, $p = 0.4933$, Fig. 4). At both depth strata, the mean SL was significantly greater in the 2000s than in the 1990s (ANOVA, $F = 46.0473$, df = 1, $p < 0.0001$, Fig. 4). A significantly greater proportion of individuals < 90 mm was detected in the 1990s than in the 2000s ($\chi^2 = 14.4034$, df = 1, $p = 0.0001$, Fig. 5); the proportion of individuals > 114 mm was significantly greater in the 2000s than in the 1990s ($\chi^2 = 45.7073$, df = 1, $p = 0$, Fig. 5). Mean live abalone SL was significantly lower in the 1990s (106.7–108.5 mm, 95% CI) than the SL of 1990s mortalities (110.4–115.7 mm, 95% CI), 2000s mortalities (113.7–119.9 mm, 95% CI), and 2000 live abalone (113.0–116.2 mm, 95% CI; ANOVA, $F = 24.5405$, df = 3, $p << 0.0001$, Tukey's test, Fig. 6).

Fig. 2. Exponential decay of counts of pinto abalone (*Haliotis kamtschatkana*) found below 4.3 m MLLW (mean lower low water) ($y = 4E + 139e^{-0.155t}$, open circles, 946 m^2) vs. abalone found above 4.3 m MLLW ($y = 2E + 51e^{-0.056t}$, solid circles, 1296 m^2) on solid rock substrate surveyed from 1992 to 2006 in the San Juan Islands. Trajectories (slopes) are significantly different ($p = 0.0029$).



Discussion

At all sites, we observed (*i*) declines in overall abundance from the 1990s to the 2000s, (*ii*) current population densities below the suggested Allee threshold (Berec et al. 2007) level of 0.15 abalone·m^{-2} (Babcock and Keesing 1999), and (*iii*) very few emergent recruits. Reasons for the declines are elusive, but hypotheses have ranged from recreational overfishing prior to fishery closure and postclosure poaching to environmental stressors such as high temperature, low salinity, or sedimentation (e.g., Vilchis et al. 2005). Some combination or all of these factors may have contributed to initial declines; it is likely that multiple Allee effects (sensu Berec et al. 2007) generated first by increased exploitation rates relative to population levels and subsequently by decreased fertilization success led to the apparent recruitment failure observed at the index stations.

The density of abalone declined significantly between the

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Fig. 3. Densities of *Haliotis kamtschatkana* at 10 index stations in the San Juan Island Archipelago, Washington State, from 1992 to 2006. Horizontal broken lines indicate the Allee threshold of 0.33 to 0.15 abalone·m^{-2}. Washington Department of Fish and Wildlife closed the fishery following the 1994 survey.

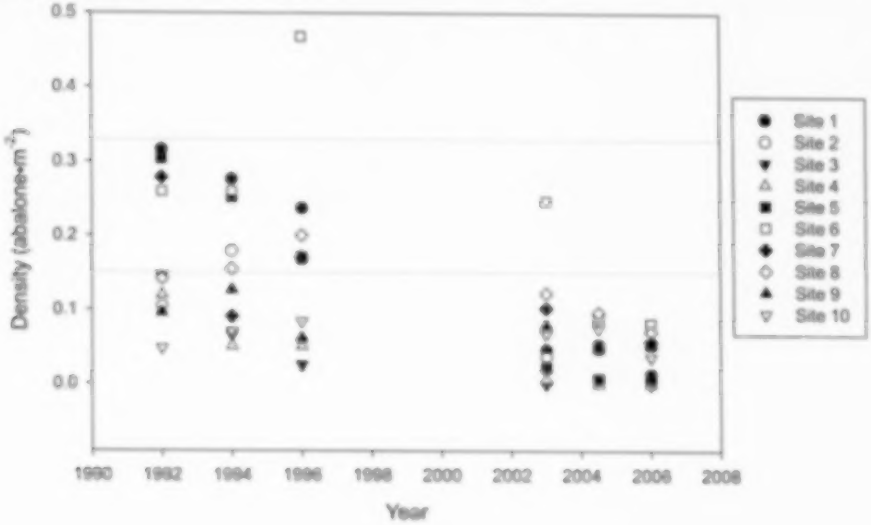


Fig. 4. Mean shell lengths of *Haliotis kamtschatkana* observed during six surveys of 10 index stations in the San Juan Island Archipelago, Washington State, during the 1990s (shaded bars) and 2000s (open bars). Depth strata boundary was at 4.3 m MLLW (mean lower low water). Error bars represent confidence intervals; letters indicate detected differences ($p < 0.0001$).

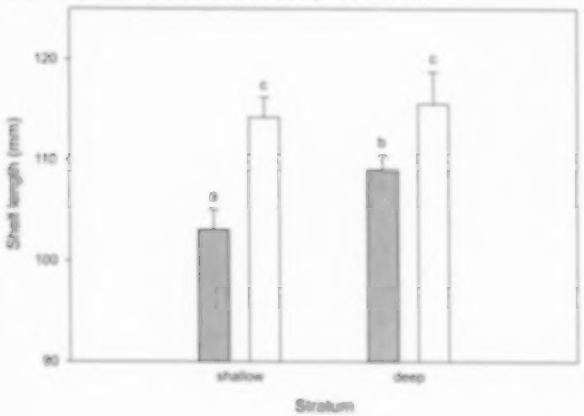
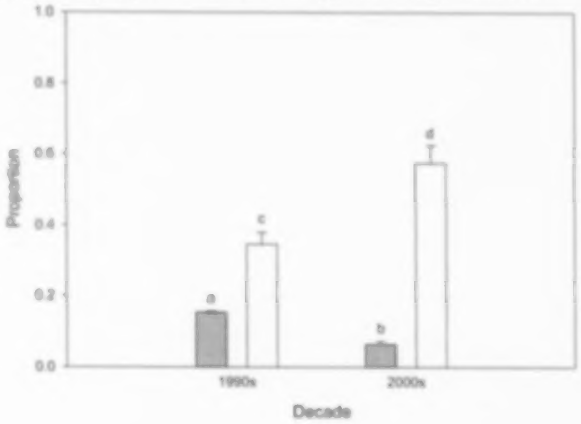


Fig. 5. Proportions of *Haliotis kamtschatkana* observed at index stations in the San Juan Island Archipelago, Washington State, with shell length < 90 mm (shaded bars) and > 114 mm (open bars), per decade. Error bars represent central confidence intervals. Differences denoted by letters are highly significant (two-sample test for equality of proportions with continuity correction, $p < 0.0001$).



1990 and 2000 surveys. The greater decline of deep stratum abalone suggests that initial declines may have been the result of fishing activity. Although abalone were generally larger in the deeper stratum (4.2–7.9 m) in the 1990s, this habitat was also more easily accessible to divers because it lacked the heavier macroalgae cover found in the shallower areas. Larger animals may have been preferentially selected from 1992 through the 1994 surveys. The sport fishery was not closed until after our 1994 survey was completed. Data from Bargmann (1984) and Gesselbracht (1991) suggest that sport harvest, centered in the SJA, may have been as high as 40 000 individuals per year. This level of effort may have been too aggressive for populations of abalone in the SJA. The significant decline of pinto abalone density despite complete closure of the fishery in 1994 is not unique to Washington State. Similar results have been documented in pinto abalone in British Columbia, Canada, where despite closure of both commercial and recreational fishing in 1990, declines continued (Sloan and Breen 1988; Tomascik and Holmes 2003).

Our length frequency data reveal a significant increase in the mean length of abalone. Although initial declines may have been a result of overexploitation, the length increase provides evidence for recruitment failure as the mechanism

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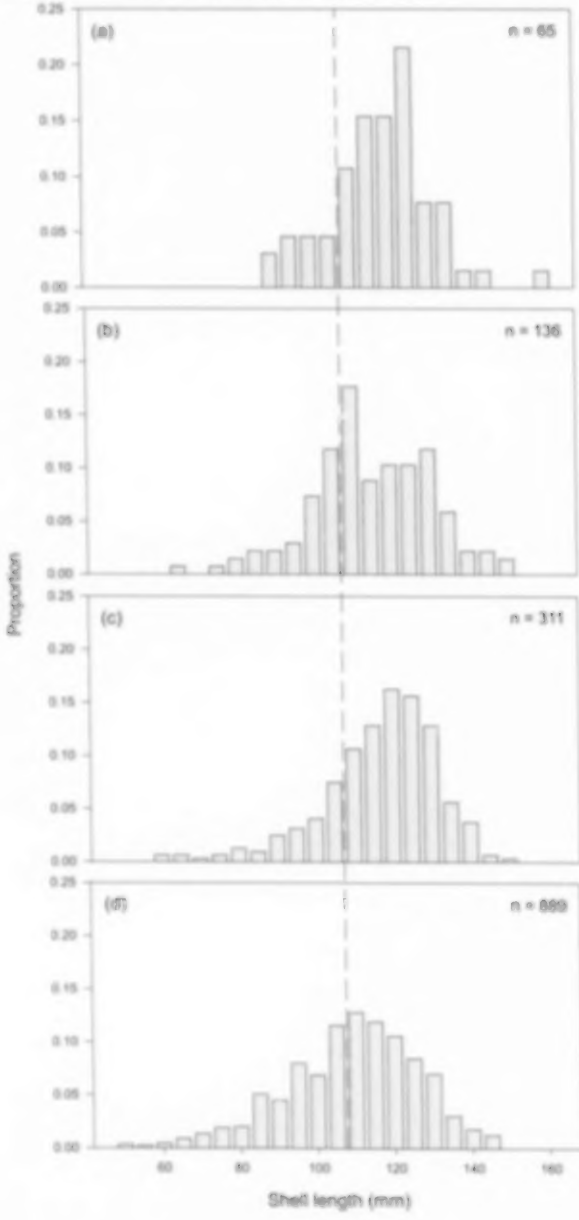

Fig. 6. Differences in length frequencies of *Haliotis kamtschatkana* observed at index stations in the San Juan Island Archipelago, Washington State, including all mortalities found in the 2000s (*a*) and the 1990s (*b*), and all live abalone observed in the 2000s (*c*) and the 1990s (*d*). The mean live abalone 1990s shell length (107.6 mm) is indicated by the broken line.

for continued declines. While emergent abalone < 90 mm comprised 16% of individuals in the 1992, 1994, and 1996 surveys, they had fallen to less than 6% of the population from 2003 to 2006. At two sites (3 and 4), very few emergent abalone were observed in the 1990s, preceding the observation of extirpation of these sites in the 2000s. In addition to the observed recruitment deficit, the increase (+8.1 mm) in mean SL of abalone between the 1990s and the 2000s suggests that abalone in the SJA are aging without replacement. Davis et al. (1996) observed a similar trend in length frequencies of endangered white abalone (*Haliotis sorenseni*) (Federal Register 66(103) 50 CFR Part 224, May 29, 2001) and suggested that the observed recruitment failure was a key factor in the demise of white abalone populations.

The most parsimonious explanation for our data is a mating-related Allee effect, or recruitment failure in response to depressed population density (Allee et al. 1949; Stoner and Ray-Culp 2000). In broadcast-spawning, sedentary invertebrates such as abalone, Allee thresholds of >0.33 to 0.15 individuals-m^{-2} (Shepherd and Partington 1995, Babcock and Keesing 1999) have been suggested as necessary for successful fertilization in some haliotides (Pennington 1985). Following closure of the fishery in 1994, densities of abalone at 50% of our index sites were at the suggested 0.15 individuals-m^{-2} Allee threshold, and one index site was above the threshold. By 2006, densities at all index stations were well below 0.15 individuals-m^{-2}; our sites experienced a 77% decrease in overall abundance over the period of the study. Although specific Allee thresholds have not been determined for pinto abalone, the density data from the early 1990s suggest that higher Allee thresholds may exist for *Haliotis kamtschatkana* in the SJA than those described for other abalone species. Densities at all of our sites may have already been too low to promote fertilization success and recruitment at the start of our study. Wells and Mulvay (1995) showed that overexploitation combined with fertilization inefficiency led to the eventual extirpation of *Tridacna gigas* in areas of the south Pacific. In abalone, fertilization inefficiencies could be exacerbated by a tendency toward episodic spawns (Tegner et al. 1989; McShane 1992; Shepherd and Daume 1996). Although early declines documented in our study could have been the result of fishing, continued declines suggest recruitment failure as the likely cause.

The increase in mean SL counters the notion that the observed declines are a result of continued illegal harvest, since it is unlikely that poachers would target smaller abalone. Conversely, it is reasonable to expect that poachers would remove larger abalone, as occurs during a legal fishery (Maliao et al. 2004). At Van Damme State Park in California, where only free diving is allowed, 16% of red abalone (*Haliotis rufescens*) exceeded the legal sport size limit in the shallow, accessible stratum (<8.4 m), whereas 43% exceeded legal sport size in the deep stratum (Karpov et al. 1998). If pinto abalone declines in the SJA were due to illegal harvest, we would expect a length frequency shift to the left (smaller), similar to the results of Karpov et al. (1998) and Maliao et al. (2004); instead, we observed the opposite.

The possibility also exists that our sampling occurred during an extended period of low recruitment in the SJA and that the declines in the proportions of emergent abalone observed in our study represent only part of a longer population cycle. However, no recruitment pulse is evident in any of the index site length frequency histograms over the 14-year survey period; recruitment at a greater frequency than observed is likely necessary for a species with an assumed 25- to 50-year life expectancy.

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Other explanations for the declines include early mortality, predation, competition, inbreeding depression, disease, and habitat loss. For example, Tegner et al. (2001) found sea surface temperatures correlated with decreased landings of red abalone, and Tomascik and Holmes (2003) showed that predation might be an important factor in limiting pinto abalone recovery. We observed no correlation between proximity to freshwater inputs, and to our knowledge there has been no loss of suitable rocky habitat. However, there has been a slight increase in temperature and decrease in salinity in the north SJA (Masson and Cummins 2004); regional sea surface temperatures in the 1990s were the warmest in recent history (Strom et al. 2004). Temperature has been shown to both directly (e.g., disease expression) and indirectly (e.g., food availability) affect abundance in other abalone species (e.g., Vilchis et al. 2005). An effect of temperature increase on pinto abalone would be more likely observed in the shallow stratum, yet our data show that abundance of pinto abalone declined most rapidly in the deep stratum, suggesting other influences on abundance. Salinity, rather than temperature, may be exacerbating recruitment failure by reducing larval and postlarval survival (Bouma 2007), but more studies on temperature and salinity effects on pinto abalone abundance are necessary. No disease impacting wild pinto abalone has been reported in Washington State. However, *Labyrinthuloides haliotidis*, a protistan parasite lethal to juvenile abalone, caused catastrophic losses of cultured pinto abalone in British Columbia, Canada, in the early 1980s (Bower 2003). A disease screening of 15 wild adults collected from the SJA in 2003 and 60 cultured juveniles taken from our research hatchery in 2007 revealed no signs of disease (C. Friedman, personal observation). A disease that affects only young wild abalone remains a possibility, but no work has been done to test this hypothesis. In addition, no evidence for inbreeding depression was found in recent genetic surveys of pinto abalone using DNA microsatellites (Washington State: K. Naish, K. Straus, and C. Friedman, unpublished data; British Columbia, Canada: Withler et al. 2003). Abiotic environmental change, increased predation, competition, and early mortality remain hypotheses to be tested in future studies.

Correlations between presence of sea urchins and abalone abundance have been demonstrated with several abalone species, including the pinto abalone. Sea urchins may keep large areas of habitat open and available for both growth of CCA and settlement of larval pinto abalone. In addition, spine canopies and home scars may serve as nursery habitat for juvenile abalone. Rogers-Bennett and Pearse (2001) observed an association of juvenile red abalone and purple urchins (*Strongylocentrotus purpuratus*) in California (but see Karpov et al. 2000), and Tomascik and Holmes (2003) observed higher densities of pinto abalone emergent recruits in areas inhabited by red urchins (*Strongylocentrotus franciscanus*) in the Broken Group Islands of British Columbia. A similar positive relationship appears to exist between densities of red sea urchins and pinto abalone in the SJA. Because of fishing pressure, many of the locales in our study have much lower abundances of red urchins than observed in the 1980s and early 1990s (Pfister and Bradbury 1996; M. Ulrich, WDFW, 600 Capitol Way North, Olympia, WA 98501, USA, unpublished data). The paucity of small and recruit-size abalone observed in our study may, in part, be a result of a decrease in suitable CCA habitat previously made available by the presence of grazing red sea urchins; gamma aminobutyric acid, or GABA, a chemical present in crustose coralline algae, is known to induce the settlement of larval abalone (Morse et al. 1979). The relationship between sea urchin and pinto abalone density in the SJA warrants investigation.

Gascoigne and Lipcius (2004) demonstrated that Allee effects can have major consequences for population dynamics, sustainable exploitation, and management of marine invertebrate species. Because little is known about the biology of pinto abalone early life stages, a better understanding of their distribution potential, environmental tolerances, and behavior is needed. Although no relationship was observed between the rate of abalone declines and freshwater input, differences in the environments adjacent to major rivers that experience freshet events may influence larval survival, metamorphosis, and settlement, as well as growth of postlarvae, for successful recruitment to these areas. If, as we suspect, Allee effects are a major contributor to the spatial and temporal changes in pinto abalone abundance in Washington State, then these populations are unlikely to recover without intervention.

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Ghost runs: management and status assessment of Pacific salmon (*Oncorhynchus* spp.) returning to British Columbia's central and north coasts

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Abstract: The management of Pacific salmon (*Oncorhynchus* spp.) populations, which are spatially distributed across thousands of waterways in coastal British Columbia, Canada, presents considerable challenges to resource managers. We evaluated the efficacy of salmon management by Fisheries and Oceans Canada (DFO) over the past 55 years in two key areas: (*i*) the achievement of internally generated target escapement levels and (*ii*) escapement monitoring. We show that less than 4% of monitored streams ($n = 7$ of 215), which represent a small fraction of all salmon-bearing waterways ($n = 2592$), have consistently met escapement targets since 1950. During this same period, the number of streams monitored by DFO has simultaneously decreased. Further, current monitoring efforts fall short of encompassing the range of salmon diversity identified within recently designated conservation units. Importantly, we found that this erosion of monitoring effort has been biased towards dropping smaller runs that failed to meet target escapements in the previous decade. We suggest that such increasingly selective monitoring is presenting a progressively more biased evaluation of population health. In addition to fostering a "shifting baseline" syndrome, we conclude that these changes to monitoring can not provide data required for precautionary harvest management under the high exploitation levels that these runs experience.

Résumé : La gestion des populations de saumons du Pacifique (*Oncorhynchus* spp.), qui sont réparties spatialement dans des milliers de cours d'eau sur la côte de la Colombie-Britannique, présente des défis importants aux gestionnaires des ressources. Nous évaluons l'efficacité de la gestion des saumons faite par Pêches et Océans Canada (DFO) au cours des 55 dernières années dans deux domaines importants: (*i*) l'atteinte des cibles d'échappement par les processus intrinsèques et (*ii*) la surveillance de l'échappement. Nous montrons que moins de 4 % des cours d'eau suivis ($n = 7$ de 215), qui représentent une petite fraction des cours d'eau à saumons ($n = 2592$), ont régulièrement atteint leurs cibles d'échappement depuis 1950. Durant la même période, le nombre de cours d'eau suivis par DFO a simultanément décru. De plus, les efforts de surveillance actuels n'arrivent pas à couvrir l'étendue de la diversité des saumons identifiée au sein des unités de conservation récemment définies. Ce qui est important de noter, c'est que cette érosion de l'effort de surveillance a frappé de façon disproportionnée les petits groupes de montaison qui n'ont pas réussi à atteindre leurs cibles d'échappement au cours de la dernière décennie. Nous croyons que cette surveillance de plus en plus sélective fournit une évaluation toujours plus faussée de la santé des populations. En plus de produire un syndrome de « données de base changeantes », ces modifications dans la surveillance ne permettent pas d'obtenir les données nécessaires pour une gestion prudente des récoltes dans les conditions de forte exploitation que connaissent ces groupes de montaison.

[Traduit par la Rédaction]

Introduction

Salmon management presents complex biological, economic, and political challenges (Wright 1981; Hilborn and Luedke 1987). In British Columbia (BC), resource managers rely heavily on stock assessment programs to guide their decision-making processes. For example, annual enumeration monitoring of spawning salmon is thought to provide critical information on stock status, trends, and productivity required to inform harvesting decisions and conservation plans (English et al. 2006). BC's Pacific Fisheries Resource Conservation Council (PFRCC 2004) considers consistent information on stock assessment a top management priority in successful salmon conservation. In contrast, ad hoc enumeration plans are thought to be inadequate to provide the quality of data needed to conserve salmon populations under heavy fisheries pressure (Routledge and Irvine 1999; PFRCC 2004; English et al. 2006).

Salmon (*Oncorhynchus* spp.) populations in BC and the entire Northeast Pacific have been, and continue to be, heavily exploited (Schmidt et al. 1998; Finney et al. 2000; English et al. 2006). They have disappeared from 40% of their historic spawning range in the continental US (Nehlsen et al. 1991) and are considered extinct in 142 watershed systems throughout BC (Slaney et al. 1996). Commercial catches in BC between 1995 and 2005 were the lowest on record (Walters and Korman 1999*a*; DFO 2008*a*). Additionally, the number of stocks contributing to this catch is also

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declining, shifting over the decades from many runs of diverse size to fewer large runs (Walters and Cahoon 1985; Wood 2001).

Under such exploitation pressures, stream-specific management escapement goals have been created by Fisheries and Oceans Canada (DFO) — the federal government agency responsible for salmon management in BC — with the aim to ensure adequate numbers of spawning salmon (Walters and Korman 1999*b*). These target escapements are based largely on professional judgement of habitat capacity, historic return records, and number of fish needed to adequately seed spawning grounds (Goruk and Winther 1992). Moreover, designed to maximize salmon production, these target escapements are considered widely relevant today (Walters et al. 2008). A primary strategy used by DFO to measure management performance relies on setting target escapement goals and detecting trends in abundance relative to these targets (Walters and Korman 1999*a*; DFO 2007). The ability to meet these escapement targets, therefore, provides a straightforward and objective way to evaluate whether basic management strategies by DFO have been achieved (Goruk and Winther 1992).

We have two objectives in assessing salmon management in BC by DFO. We first evaluate the frequency by which escapement targets have been achieved over the past half-century. In doing so, we thus also provide a contemporary assessment of the general status of salmon populations in BC relative to historical benchmarks. Second, we examine monitoring efforts by DFO over the same period. Specifically, we assess the changes to monitoring efforts over time and examine which factors have led to the considerable decrease in annual enumeration we document.

Materials and methods

We examined escapement data for BC's central and north coasts (DFO management areas 3 through 10; Fig. 1) between 1950 and 2005. This region consists of numerous isolated islands and mainland watersheds. Although this area includes several watersheds unmodified by roads or logging, fisheries exploitation and hatchery supplementation have occurred for more than a century. We assessed five commercially important salmon species: *Oncorhynchus tshawytscha* (Chinook), *Oncorhynchus kisutch* (coho), *Oncorhynchus nerka* (sockeye), *Oncorhynchus keta* (chum), and *Oncorhynchus gorbuscha* (pink). Given their consistent 2-year life cycle, we separated pink into even and odd years in analyses.

Trends in achieving target escapements

We assessed escapement trends in the study area for all years between 1950 and 2005 using DFO BC16 reports (DFO 1992) and DFO's Salmon Escapement Database System (NuSEDS; DFO 2008*b*). Although the accuracy of estimates contained within these databases has been questioned by some (e.g., Irvine and Nelson 1995), no alternative or complementary data are available.

We classified spawning records into three categories: indicator streams (sites defined by DFO and enumerated consistently over time); nonindicator streams (inconsistent enumeration of spawners); and streams without information (spawners confirmed, yet no enumeration data collected). Although DFO infers general trends in abundance and productivity from indicator streams, many of these sites were not visited annually (particularly in recent years; see below). Accordingly, we described the monitoring of an indicator stream as unknown when enumeration information was gathered for less than 50% of the years within any one decade.

Using only indicator streams (and excluding unknown decades), we averaged annual escapement returns for each decade and compared them to management target escapement (MTE) values established by DFO. For each decade, we classified stream escapements as meets target (80% or more of the spawner target was met), depressed (40%–79%), and very depressed (<40%). Additionally, we examined the percentage of newly designated conservation units (CUs; DFO 2008*c*) for each species that was minimally monitored. We considered a CU meeting this minimal criterion if it contained a minimum of one enumerated stream during the period 2000–2005.

Evaluation of escapement monitoring

We were interested in which factors might influence changes in monitoring effort over the last half century. Specifically, we observed that fewer indicator streams were monitored each decade, but we did not know which factors were associated with monitoring cessation. Accordingly, we formed a priori hypotheses relating salmon run characteristics to the probability of runs ($n = 215$) being dropped from monitoring effort. Run characteristics included (*i*) run status during the previous decade (status; either meets target or not (i.e., depressed or very depressed)), (*ii*) potential run size (size; management target escapement values), and (*iii*) salmon species (species). From these hypotheses, we developed a set of candidate generalized linear regression models (binary logistic form). A Hosmer–Lemeshow goodness-of-fit statistic based on the global model showed the data did not depart from a logistic form ($P = 0.491$). For each model, we calculated Akaike's information criteria, following the formula: $AIC_c = -2(\log \text{likelihood}) + 2K + 2K(K + 1)/(n - K - 1)$, where K is the number of parameters and n is the number of indicator salmon runs. We then evaluated ΔAIC_c to select the best approximating model(s) and made appropriate inference, using $\Delta AIC_c < 4$ to describe the top model set. Finally, we summed Akaike weights (ω_i) across the top model set for each variable to rank them by importance (Burnham and Anderson 1998; Anderson et al. 2001). Tests were performed using SPSS 13.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Trends in achieving target escapements

Evaluation of all species showed that the achievement of target escapements varied considerably by species over the last 55 years, but overall that less than 4% of monitored streams consistently met their decadal MTEs. Currently (i.e., between 2000 and 2005), and only when unknown systems that were dropped from monitoring efforts are omitted, coho and pink salmon are the only species to meet target escapements at more than 50% of their indicator streams (Table 1). Accordingly, under typical evaluation perspectives, the status of odd-year pink (72%; $n = 36$), even-year pink

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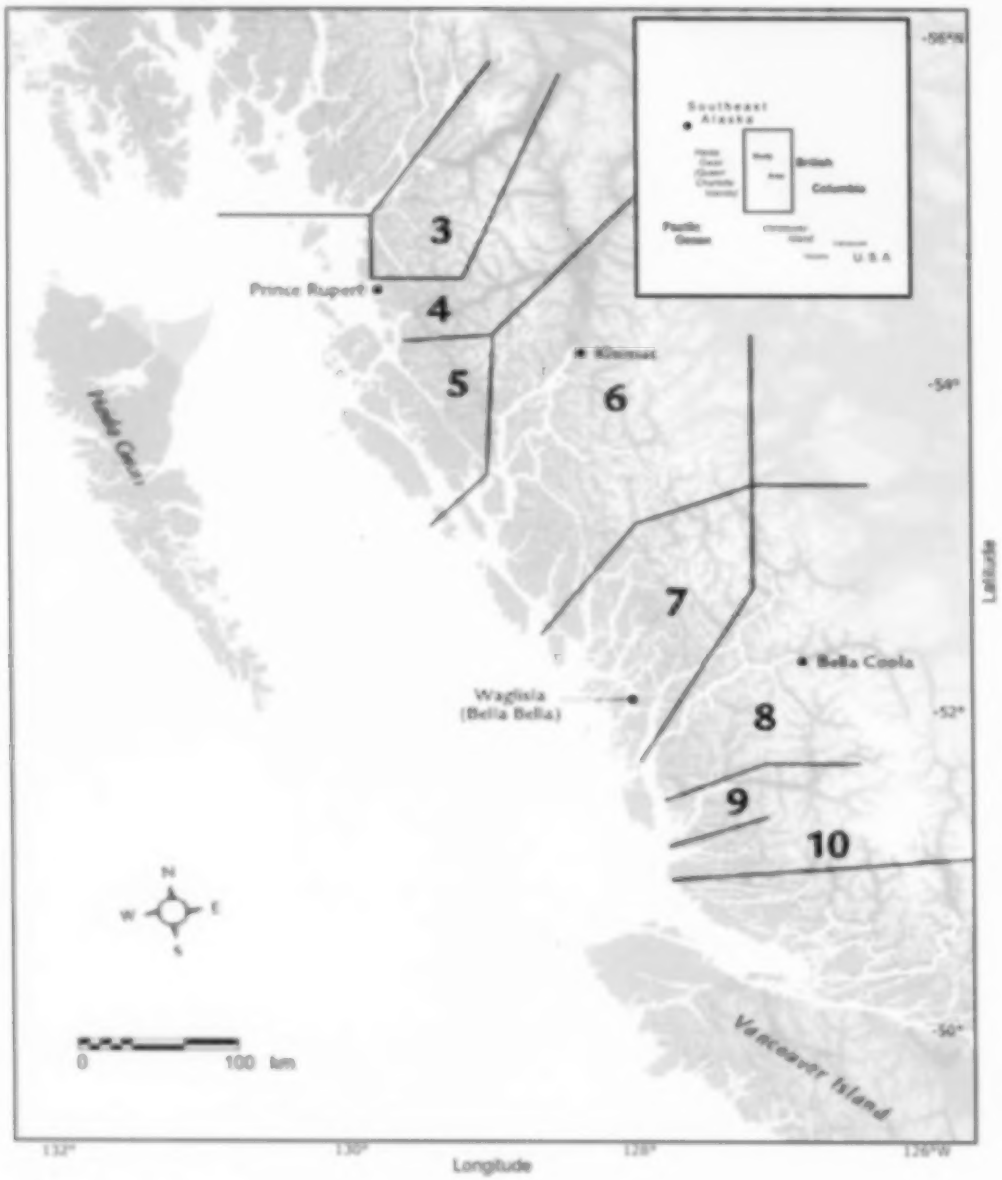
Fig. 1. Study area, encompassing Fisheries Management Areas 3–10, for Pacific salmon (*Oncorhynchus* spp.) returning to British Columbia's central and north coasts during 1950–2005.



(66%; $n = 34$), and coho (63%; $n = 5$) appears to show relatively high levels of meeting escapement targets. Conversely, for Chinook, chum, and sockeye, runs currently classified as depressed or very depressed exceed 50% (Table 1).

Evaluation of escapement monitoring

DFO's BC16 reports identify 2592 runs of the five commercially harvested salmon species on BC's central and north coasts (Table 2). We found 30% of these runs ($n = 768$) were enumerated at least once between 1950 and 2005. Consistent data on abundance trends, however, exist for only 8% ($n = 215$). Although coho streams are the most numerous among species, knowledge of spawner abundance is the poorest (<4%, $n = 33$ of total coho — $n = 891$). Chinook and sockeye have the greatest proportion of runs classified as indicator streams (13% each), followed by chum and pink (10% each). Though differing by species, streams with sufficient escapement data to evaluate trends have decreased from a high of 210 (98%) in 1980 to a low of 137 (64%) in 2005 (Fig. 2). Annual enumeration in the present decade is low for all species, but is particularly low for coho at only 2% ($n = 7$). Finally, our examination of

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Table 1. Average escapement by species and decade and associated status categories for indicator streams ($n = 215$) of spawning Pacific salmon (*Oncorhynchus* spp.) returning to British Columbia's central and north coasts during 1950–2005.

Species	No. of catalogued spawning runs	No. of classified indicator streams*	No. of indicator streams* annually enumerated 2000–2005	No. of CUs† identified	Percent CUs† monitored by indicator streams*
Chinook	215	27	6	22	41
Coho	891	33	6	15	40
Sockeye	320	40	8	119	13
Chum	492	49	8	16	75
Pink	674	66	27		
Odd-year				5	88
Even-year				8	80
Total	2592	215			

*Indicator streams are salmon runs consistently enumerated, as classified by Fisheries and Oceans Canada.
†Enumeration of as few as one indicator stream in a conservation unit (CU) is classed as monitored.

Table 2. Synopsis of catalogued spawning runs, indicator streams, and current monitoring effort for Pacific salmon (*Oncorhynchus* spp.) returning to British Columbia's central and north coasts during 1950–2005.

		Species					
Decade	Assessment	Chinook	Coho	Sockeye	Chum	Pink, even	Pink, odd
1950s	Escapement	81 635	194 286	1 214 571	480 042	1 539 823	1 870 768
	Meets target (%)	19	15	25	22	23	14
	Depressed (%)	11	21	28	37	17	15
	Very depressed (%)	30	42	30	37	50	61
	Unknown (%)	41	21	15	4	11	11
1960s	Escapement	71 320	192 022	1 529 056	472 922	4 339 656	2 034 378
	Meets target (%)	15	15	18	18	44	20
	Depressed (%)	11	21	40	41	21	24
	Very depressed (%)	48	36	40	39	26	50
	Unknown (%)	26	27	3	0	9	6
1970s	Escapement	57 216	116 404	1 949 039	523 603	3 445 347	1 982 724
	Meets target (%)	0	9	23	22	52	15
	Depressed (%)	22	15	38	39	24	42
	Very depressed (%)	67	64	40	39	20	36
	Unknown (%)	11	12	0	0	5	6
1980s	Escapement	77 077	104 189	2 841 318	512 508	3 648 158	4 257 666
	Meets target (%)	7	9	25	12	41	42
	Depressed (%)	19	33	40	45	33	33
	Very depressed (%)	67	48	35	43	26	24
	Unknown (%)	7	9	0	0	0	0
1990s	Escapement	109 286	40 858	2 648 937	615 140	3 644 587	3 367 981
	Meets target (%)	11	15	20	16	29	29
	Depressed (%)	22	9	23	20	41	32
	Very depressed (%)	56	9	50	57	26	32
	Unknown (%)	11	67	8	6	5	8
2000s	Escapement	62 116	62 246	1 407 130	716 146	3 697 642	4 769 329
	Meets target (%)	7	18	20	20	51	54
	Depressed (%)	15	3	5	27	11	17
	Very depressed (%)	26	6	48	24	21	6
	Unknown (%)	52	73	28	29	17	23
Management goal*		210 175	444 000	2 218 250	1 092 000	4 384 000	4 384 000

*Management goal is Fisheries and Oceans Canada annual escapement target (MTE).

monitoring efforts within CU areas showed a range of minimal representation (at least one stream monitored) from 13% (sockeye) to 88% (odd-year pink), with an average of 28% across species (Table 2).

Model selection and multimodel inference suggested that status and size are the best predictors of monitoring cessation (Table 3). Specifically, the probability of runs being dropped from monitoring increased primarily with depressed

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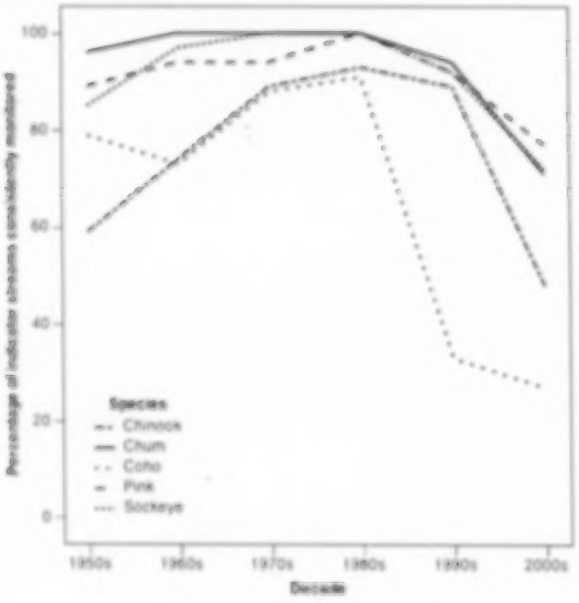
Table 3. Top generalized linear regression (logistic form) model set to predict the monitoring cessation of indicator salmon runs of British Columbia's central and north coasts.

Model	K	Deviance	AIC_c	ΔAIC_c	ω_i	Nagelkerke R^2
Status + size + (status × size)	4	260.872	269.327	0.000	0.434	0.121
Status	2	267.315	271.448	2.122	0.150	0.083
Status + species	3	265.239	271.509	2.182	0.146	0.096
Status + size	3	265.365	271.635	2.308	0.137	0.095
Status + size + species	4	264.209	272.664	3.337	0.082	0.102

Note: Nagelkerke R^2 values are always less for logistic models than for linear models. Model structure, deviance, corresponding ΔAIC_c, Akaike weight (ω_i), and Nagelkerke R^2 are included. Status is run status, size is run size, and species is salmon species.

Fig. 2. Decadal trends in monitoring effort for Pacific salmon (*Oncorhynchus* spp.) indicator streams on British Columbia's central and north coasts during 1950–2005.



or very depressed status, but less so with run size and species; all five models in the top model set (0–4 ΔAIC_c) contained status (Table 4). Summing the Akaike weights across top models ranked the variable status ($\Sigma\omega_i$ = 0.949) higher than size, status × size, and species by factors of 1.45, 2.19, and 4.16, respectively.

Discussion

Trends in achieving target escapements

Escapement enumeration is an important tool used by DFO in the assessment of abundance and subsequent establishment of harvest levels (Walters and Korman 1999*b*; English et al. 2006). Escapement data, ideally, should be continually compared with benchmark values to form the foundation of salmon management. Data we summarized, which span nearly six decades, show that management has repeatedly not met DFO's own target levels. This resulted in diminished runs for all species in nearly every decade,

Table 4. Summed Akaike's information criteria (AIC) weights ($\Sigma\omega_i$) across the top model set to rank parameters by relative importance in predicting the cessation of monitoring efforts of indicator salmon runs on British Columbia's central and north coasts.

Parameter	$\Sigma\omega_i$	Direction of association
Status	0.949	Diminished status in previous decade
Size	0.653	Smaller runs
Status × size	0.434	Diminished status when run size is small
Species	0.228	Order of species: coho, chum, pink, sockeye, Chinook

Note: Status is run status, size is run size, species is salmon species, and direction of association describes in which manner the parameters are associated with monitoring cessation.

and as we describe below, was followed by increasingly selective monitoring programs that cannot accurately evaluate the health of salmon populations on BC's coast.

We acknowledge that meeting target escapements is an undoubtedly difficult task for managers given the complexity of Pacific salmon fisheries and the myriad of ecological factors that conspire to affect returning spawner numbers (Wright 1981; Hilborn and Luedke 1987). We point out, however, that MTEs were goals created by DFO, which by setting exploitation levels, would influence (at least in part) the health of salmon populations in BC (Goruk and Winther 1992; Walters and Korman 1999*b*; Walters et al. 2008).

Meeting internal management objectives is directly related to exploitation levels. Although climate and ocean survival likely play substantial roles, multiple lines of evidence suggest that overexploitation may be the greatest cause of salmon declines across the Northeast Pacific (Schmidt et al. 1998; Finney et al. 2000; Schindler et al. 2005). A recent report commissioned by DFO estimated that 48% of salmon runs in management areas 3 to 10 were either "highly exploited" or of "conservation concern" (English et al. 2006). For example, exploitation rates of Skeena River coho ranged from 40% to 90% between 1946 and 1997 (DFO 1999), resulting in an average 74% reduction in spawners over this period and subsequent fishery closure in 1998. Exploitation proportions of this magnitude have repeatedly resulted in decline and collapse of fisheries globally (Myers et al. 1996; Pitcher 2001; Belgrano and Fowler 2008). Coho numbers in the Skeena River system have steadily increased since this 1998 closure, and although increased marine survival likely contributed, the rebound of coho clearly demonstrates the efficacy of reducing fishing pressure as a

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straightforward management prescription to rebuild diminished runs.

Evaluation of escapement monitoring

Not only are management targets consistently not being met, but also monitoring efforts are constantly eroding over the decades. As our data indicate, this handicaps current and future assessments of management and salmon population status. A constantly eroded effort and one biased to larger, healthier runs falls far short of the strategic effort required for informed fishery and salmon conservation decisions. A detailed report by the PFRCC (2004) concluded that the first priority in salmon conservation is consistent information (i.e., collected every year). Moreover, this current monitoring program is inadequate compared with specific recommendations made by an independent team commissioned by DFO (English et al. 2006), who called for a minimum of 407 streams enumerated yearly in this area, with an additional 152 runs enumerated every 2–4 years. Any effort less than this inhibits DFO's ability to accurately or precisely assess regional trends and can not provide the quality of data needed to conserve salmon populations under heavy fisheries pressure (PFRCC 2004; English et al. 2006). This is especially relevant for small runs (Routledge and Irvine 1999), which are disproportionately dropped from monitoring programs. If limited DFO resources earmarked for fieldwork are responsible for the monitoring patterns we document, we recommend that these responsibilities be increasingly shared with First Nations and nonprofit organizations, as has begun (e.g., Temple 2007). By sharing this responsibility, however, we suggest that these participants become increasingly involved in management decisions (i.e., setting exploitation limits).

How do our data on monitoring efforts relate to the most contemporary of management action plans? DFO recently published its CUs for wild salmon that identify the genetic and ecological scales at which salmon should be conserved and managed (DFO 2008*c*). It follows that these CUs should be adequately monitored. We show, however, a generally low monitoring effort, even applied at this scale. These data reinforce our conclusion that current monitoring efforts are inadequate to conserve salmon populations and indeed question the value of CUs, if largely unmonitored, as a new management tool for conservation.

Although budget shortfalls might contribute to monitoring declines in general (Slaney et al. 1996; PFRCC 2004), we present here some insight into how these decisions were made. Our data show that salmon runs that did not meet target escapements in the previous decade and were historically small were those most likely to be dropped from monitoring efforts. The consequence is an increasingly biased view of population health. Monitoring only healthy runs offers an uninformed and potentially perverse evaluation of regional population health. For example, the percentage of runs classified as depressed or very depressed during 2000–2005 for all species combined is 35%. Yet, if the number of runs classified likewise in the previous decade (and subsequently dropped from current monitoring) were to be included, the percentage increases to 72%. This can be likened to a "shifting baseline" (Pauly 1995) in the context of monitoring efforts.

Conservation implications

Why should managers be concerned with a monitoring effort increasingly biased towards large, healthy runs? Given that small spawning streams (*i*) far outnumber larger systems in this region (PFRCC 2004; English et al. 2006), (*ii*) are for the large part unmonitored, and (*iii*) those that are (or were) have (or had) a higher probability of monitoring cessation, several conservation implications arise.

Combined, small streams likely contribute disproportionately to ecosystem productivity and wider ecological and evolutionary processes. Coastal systems in this region are limited in nitrogen and phosphorous, which salmon carcasses provide, supplying important nutrients for aquatic and terrestrial primary productivity (Hyatt and Stockner 1985; Stockner and MacIsaac 1996). Small streams likely better enhance productivity of riparian communities compared with large streams by increasing carcass availability for terrestrial consumers and nutrient transporters (Quinn and Kinnison 1999; Quinn et al. 2003). Also, small streams facilitate carcasses flushing back into the ocean, providing estuarine nutrients that may serve as a positive feedback mechanism for salmon production (Fujiwara and Highsmith 1997; Gende et al. 2002). Moreover, wide distribution of such allochthonous resources can increase ecological and phenotypic diversity among individuals in receiving populations of terrestrial consumers (e.g., Hocking et al. 2007; Darimont et al. 2008*a*, 2008*b*).

Additionally, given current exploitation pressures and the prevailing mixed-stock harvest strategies, such biased monitoring might lead to harvest management that risks the extirpation of small runs. A notable example is Skeena River sockeye, for which harvest levels are based on the abundance returning to the Babine River spawning channels, a large and enhanced run. As a result, all other (and smaller) runs have declined substantially (Wood 2001; Walters et al. 2008). If current exploitation levels and monitoring efforts remain unchanged, waterways along BC's central and north coast might host only ghost runs — diminished or extirpated systems that once flourished with salmon.

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Terrestrial carbon contribution to lake food webs: could the classical stable isotope approach be misleading?

Marie-Elodie Perga, Martin Kainz, and Asit Mazumder

Abstract: Carbon stable isotope analyses have been widely used to estimate terrestrial carbon contribution to lake secondary production. In such approaches, phytoplankton is tacitly assumed as a single, isotopically homogenous source. Such assumption might be valid if (*i*) zooplankton do not feed selectively on specific algal taxa within bulk phytoplankton, or (*ii*) although zooplankton do feed selectively, the variability in the $\delta^{13}C$ values amongst the different algal taxa is small compared with the variability between the $\delta^{13}C$ values of bulk phytoplankton and terrestrial end-members. In a summer field study of six coastal lakes of British Columbia, Canada, we tested these assumptions using gut pigments and stable isotope measures on zooplankton and particulate organic matter. Results revealed that filter-feeding cladocerans positively selected cryptophytes, shown to be substantially ^{13}C-enriched compared with bulk phytoplankton and even with the terrestrial end-member. Comparing a classical two-source mixing model and a model that accounted for algal isotopic heterogeneity, we showed that the use of a classical two-source model can result in an overestimation of terrestrial contribution to zooplankton secondary production.

Résumé : Les analyses en isotopes stables du carbone ont été largement utilisées pour estimer la contribution du carbone d'origine terrestre à la production secondaire des lacs. Dans ces approches isotopiques, le phytoplancton est tacitement considéré comme une source unique et isotopiquement homogène. Cette supposition peut être valide sous l'hypothèse selons laquelle (*i*) le zooplancton ne se nourrit pas sélectivement de certains taxons algaux au sein du phytoplancton ou (*ii*) même si le zooplancton se nourrit de façon sélective, la variabilité des $\delta^{13}C$ entre les différentes taxons algaux n'excède pas la variabilité des $\delta^{13}C$ entre la source algale globale et la source terrestre. Nous avons testé ces hypothèses au cours d'une étude de terrain menée sur six lacs côtiers de Colombie-Britannique (Canada) en été en analysant les pigments du tube digestif et la composition isotopique du zooplancton et de la matière organique particulaire. Les résultats montrent que les cladocères filtreurs sélectionnent les cryptophytes, dont la composition isotopique est particulièrement enrichie en ^{13}C par rapport au phytoplancton total et même à la matière organique d'origine terrestre. En comparant les estimations issues d'un modèle de mélange classique à deux sources à celles d'un modèle prenant en compte l'hétérogénéité isotopique des algues, nous montrons que le modèle classique à deux sources conduit à une surestimation de la contribution du carbone terrestre à la production secondaire zooplanctonique.

Introduction

Recent stable isotope studies brought new perspectives to the ecological importance of terrestrial subsidies in fuelling aquatic ecosystems (Bouillon et al. 2003; Carpenter et al. 2005). Since crustacean zooplankton play a key role in transferring organic matter from the base of the pelagic food web to organisms at higher trophic levels, the identification and quantification of the contribution of distinct C sources to zooplankton secondary production have been a critical research topic (Grey et al. 2000; Karlsson et al. 2003; Perga et al. 2006). In such studies, two end-members have been considered as potential primary C sources to zooplankton: allochthonous organic matter and bulk phytoplankton. Allochthonous C exhibit a rather consistent $\delta^{13}C$ signature of around –26‰ to –28‰ for C_3-type vegetation (O'Leary 1988). Phytoplankton $\delta^{13}C$ signature has rarely been measured directly (Grey et al. 2000), as phytoplankton cells are difficult to isolate from other particles (bacteria, protists, and detritus of different origins) that compose the bulk particulate organic matter (POM). However, different types of models have been developed to estimate a $\delta^{13}C$ signature for the phytoplankton end point (reviewed in Marty and Planas 2008). Once phytoplankton $\delta^{13}C$ is measured or assessed, zooplankton dependence on terrestrial- versus phytoplankton-derived C is estimated resorting to two-source mixing models, with terrestrial organic matter and phytoplankton as the two end-members. For whatever method used to obtain bulk phytoplankton $\delta^{13}C$, phytoplankton is tacitly considered as a single, isotopically homogenous dietary source. Such an approach might be valid if at

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least one of the following two hypotheses is verified: (*i*) zooplankton do not feed selectively on specific algal taxa within bulk phytoplankton, or (*ii*) although zooplankton do feed selectively, the variability in the $\delta^{13}C$ values amongst the different algal taxa is small compared with the variability between the $\delta^{13}C$ values of bulk phytoplankton and terrestrial end-members.

However, the edibility and dietary quality of phytoplankton differ among algal taxa (Ahlgren et al. 1990), and zooplankton may ingest and consequently assimilate preferentially specific algal taxa within phytoplankton. For example, copepods can select particles according to their size and nutritional quality (Kerfoot and Kirk 1991). *Daphnia* are more limited in discriminating particles on the basis of their quality as food resource, but select according to particle size (<35 μm; Burns 1968), shape (Hartmann and Kunkel 1991), and abundance (Thys et al. 2003). Secondly, algal $\delta^{13}C$ values vary according to algal size, growth rate, and species-related physiology (Takahashi et al. 1991; Burkhardt et al. 1999), and thus $\delta^{13}C$ variability among algal taxa can be substantial (Fry and Wainright 1991; Pel et al. 2003; Vuorio et al. 2006). Large variability in the $\delta^{13}C$ signatures among algal taxa concomitant to zooplankton feeding selectivity on some of these algal classes would consequently challenge the relevancy of using a single phytoplankton end-member in stable isotope studies of zooplankton C sources.

The aim of this paper was to investigate whether these two implicit hypotheses can be supported in coastal lakes of British Columbia, Canada. The first hypothesis (nonselective feeding on phytoplankton) was tested directly using algal-derived pigment biomarkers on POM and zooplankton (filter-feeding cladocerans). The second hypothesis ($\delta^{13}C$ variability amongst phytoplankton classes is smaller than the variability between the $\delta^{13}C$ values of the phytoplankton and allochthonous end-members) was tested indirectly by investigating how POM $\delta^{13}C$ was related to the following predictors: (*i*) chlorophyll *a* (Chl *a*) concentration as a proxy for phytoplankton biomass, (*ii*) phytoplankton dilution by nonphytoplanktonic cells, and (*iii*) composition of algal taxa. Testing these two hypotheses allows us to compare estimates of zooplankton allochthony computed from a classical two-sources mixing model and those from a model that accounted for phytoplankton isotopic heterogeneity.

Materials and methods

This field study was conducted in July 2005 at six coastal, monomictic lakes on Vancouver Island, Canada, as described in a previous study (Kainz et al. 2004). Shawnigan Lake (SHL; 48°37′N, 123°38′W) and Elk Lake (ELL; 48°31′N, 123°23′W) are natural lakes, and Council Lake (COL; 48°31′N, 123°40′W), Sooke Reservoir (SOL; 48°33′N, 123°41′W), Goldstream Reservoir (GOL; 48°30′N, 123°38′W), and Butchard Reservoir (BUL; 48°32′N, 123°39′W) are protected from public access. The morphometry of SHL and SOL is very similar; both lakes have a shallow (-S) and a deep (-D) basin. These lakes are circumneutral (pH ~7), with thermally stratified, well-oxygenated (>2 mg dissolved oxygen·L^{-1}) water columns (Kainz et al. 2004). In every lake, except ELL, littoral areas are very small, and frequent changes of water levels in reservoirs result in substantial bank erosion. In ELL, however, littoral areas are minor compared with total lake surface area. Zooplankton and POM were collected at the deepest point of lake basins at SHL (24 m, -S; and 49 m, -D), SOL (22 m, -S; and 70 m, -D), COL (20 m), ELL (13 m), BUL (41 m), and GOL (28 m).

Plankton sampling and sample processing

Zooplankton were sampled by vertical hauls on the entire water column using a 64 μm mesh, 50 cm diameter Wisconsin net and subsequently filtered and retained in a filter cup (200 μm mesh size). Zooplankton samples intended for pigment analyses were anaesthetized in carbonated water to prevent regurgitation (Quiblier-Lloberas et al. 1996), immediately put in the dark onto dry ice to limit organic matter degradation, and kept at cryogenic temperature (–80 °C) in the lab. Samples intended for isotope analyses were maintained in lake-filtered water overnight for gut clearance. Subsamples of zooplankton were thawed and quickly separated under dim light into the main taxa of filter-feeding cladocerans (*Daphnia* spp. and *Holopedium gibberum*) and subsequently put back at –80 °C before being freeze-dried. All samples were kept at –80 °C until further analysis.

In oligotrophic and mesotrophic lakes, during the growing season, the highest production levels often occur at or below the thermocline, referred to as deep chlorophyll maxima (DCM; Fee 1976; Gross et al. 1997). DCM are not only important because of their contribution to primary production, but also provide an important food source for zooplankton (Lampert and Grey 2003). In previous isotope studies of zooplankton feeding, POM was collected from surface waters during the growing season, when lakes were stratified (Del Giorgio and France 1996; Grey et al. 2000; Karlsson et al. 2003). We argue, however, that POM sampled at the DCM may better represent POM on which zooplankton were likely to graze than POM sampled from surface waters. DCM at each station were detected by measuring Chl *a* (vertical lake profiles) using a submersible fluoroprobe equipped with automatic chlorophyll analysis (bbe Moldaenke, Kiel-Kronshagen, Germany). POM, representing the most edible diet-size spectrum for cladocerans (<35 μm; Burns 1968) was collected at depths of the Chl *a* maxima at each sampling station using a Van Dorn water sampler. To test whether $\delta^{13}C_{POM}$ varied with depth, integrated epilimnetic water samples were also collected, using a 10 m length of Tygon tubing with a weight attached at one end, at four lake stations (SOL-S and SOL-D; SHL-S and SHL-D). POM (<35 μm) was filtered on ashed Whatman GF/F filters (0.7 μm pore size) and kept frozen in the dark at –80 °C until lyophilization.

Pigment analysis

Pigments were analyzed on POM to assess the relative biomass of major algal classes (Mantoura and Llewellyn 1983). For cladocerans, analysis of gut pigments reflects qualitatively the type of algae ingested, as algal pigments are relatively well conserved in their guts; in contrast, for copepods, carotenoids are degraded to varying degrees in their gut, and thus their gut pigments do not reflect the type of algae ingested (Quiblier et al. 1994; Pandolfini et al.

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2000). Based on this finding, our study was restricted to filter-feeding cladocerans.

Pigments were extracted and analyzed using an ion-pairing reagent solution (0.75 g tetrabutyl ammonium acetate and 7.7 g ammonium acetate in 100 mL of Easy-Pure water; Quiblier-Lloberas et al. 1996). Briefly, all samples were ground in 5 mL of 90% high-performance liquid chromatography (HPLC)-grade acetone and transferred into scintillation vials (5 mL). Vials were placed on ice in a sonication bath and sonicated for 15 min. After storage in the dark at 4 °C (12 h), samples were sonicated again for 15 min and centrifuged. The final extract (3 mL) were transferred into clean scintillation vials and evaporated under a gentle stream of N_2. Dried extracts were redissolved into an injection solution (500 µL; eluant A: 10% ion-pairing reagent in methanol by volume; Mantoura and Llewellyn 1983), and Sudan II solution (3.2 mg·L^{-1}; Sigma-Aldrich Inc., St. Louis, Missouri) was used as internal reference (Leavitt et al. 1989). Samples were loaded on an autosampler (DIONEX AutoSelect AS50) equipped with a 350 µL injection loop. The separation of pigments was performed on a Radial-Pak C18 column (0.8 cm × 15 cm, 5 µm particle size) by linear gradient elution from 100% eluant A (10% ion-pairing solution in MeOH) to 100% eluant B (27% acetone in MeOH) for 7 min, followed by 15.5 min of 100% eluant isocratic hold. Flow rate was 1.5 mL·min^{-1} and absorbance was read at 435 nm. Peak areas were converted to concentrations by the external standard calibration method using commercially available pigment standards (Dionex Canada Ltd., Oakville, Ontario) for Chl *a*, fucoxanthin, zeaxanthin, alloxanthin, and lutein.

The contribution of the four main algal types (diatoms, cryptophytes, chlorophytes, and cyanobacteria) to the sample (POM or cladocerans) Chl *a* biomass was quantified using CHEMTAX (Mackey et al. 1996), with marker pigments to Chl *a* ratios as reported elsewhere (Thys et al. 2003). These ratios were 0.606 (for fucoxanthin in diatoms), 0.281 (for alloxanthin in cryptophytes), 0.149 (for lutein in chlorophytes), 0.022 (for zeaxanthin in chlorophytes), and 0.183 (for zeaxanthin in cyanobacteria). Multiplying the average content of each marker pigment by the corresponding ratio yields the estimate of the Chl *a* biomass for the corresponding algal class per milligram of sample. Results were reported as Chl *a* equivalent per milligram of dry sample (equiv. Chl *a*·mg^{-1} dry weight).

Stable isotope analysis

Freeze-dried samples (1 mg) were placed into tin capsules and subsequently analyzed for $\delta^{13}C$, %C, and %N on a stable isotope ratio mass spectrometer (Finnigan Delta Plus Advantage). As we could not get enough matter to run replicates for all samples, we calculated the variability (reported as 1 standard deviation, SD) of $\delta^{13}C$ values for zooplankton on three pairs of replicates (0.30‰) and in POM on six pairs of replicates (0.20‰).

Because body composition, assessed from C:N ratios, can affect zooplankton $\delta^{13}C$ (Matthews and Mazumder 2005), we normalized the $\delta^{13}C$ values of zooplankton to constant lipid content; we calculated lipid-extracted $\delta^{13}C$ values using a stoichiometric model elaborated by Matthews (2006). The model uses zooplankton C:N ratio and an empirical average difference between the $\delta^{13}C$ in lipids and carbohydrates–proteins (i.e., lipid-extracted tissue; $\Delta_{C\text{-}L}$). The model parameters were measured from an annual study at SOL-D in 2002 for cladocerans. We used $\Delta_{C\text{-}L\ cladocerans} = 5.1$ (standard error (SE) = 0.33, n = 14). All the $\delta^{13}C$ values for zooplankton were reported as lipid-normalized values.

Data analysis

C:N ratios of POM, used as an indicator of phytoplankton dilution by terrestrial-derived particles (Kendall et al. 2001), were provided by the mass spectrometer (MS) results. Zooplankton feeding selectivity for some algal type was detected using Ivlev's electivity index (Ivlev 1961). Electivity of zooplankton taxa for alga type i (E_i) was calculated as

$$E_i = \frac{r_i - p_i}{r_i + p_i}$$

where r_i is the proportion of the algal class i in zooplankton guts, and p_i is the proportion of the algal class i in filtered (0.7–35 µm) algal POM (bulk measurement). The algal class i was considered as positively selected when $E_i > 0.25$ and negatively selected when $E_i < -0.25$. For $-0.25 \leq E_i \leq 0.25$, zooplankton do not exert any particular selection (Ballantyne et al. 2003).

Paired t tests were used to detect common trends in the change of the descriptors between the two seasons. We relied on nonparametric tests (Wilcoxon test) when variances were not likely to be similar (tested by Levene tests). The difference between cladocerans $\delta^{13}C$ and $\delta^{13}C_{POM}$ were reported as $\Delta\delta^{13}C_{cladocerans}$. The relationships among $\delta^{13}C_{POM}$, $\Delta\delta^{13}C_{cladocerans}$, and the various descriptors were investigated by simple correlations and eventually linear regressions when significant correlations could be detected. Kruskal–Wallis tests were used to detect significaht differences in $\Delta\delta^{13}C_{cladocerans}$ with lake trophic status.

Results

Lake and POM characteristics

All stations, except SOL-D, had deep Chl *a* maxima (i.e., phytoplankton biomass peaks at or below the thermocline (Table 1). Chl *a* concentrations at the DCM ranged from 1.7 to 29.8 µg·L^{-1}. The phytoplankton biomass of POM was dominated by diatoms and chlorophytes and (or) cyanobacteria, while cryptophytes were always a minor fraction (<25%) of phytoplankton biomass or even absent in BUL and COL (Figs. 1*a*, 1*b*). The filter-feeding cladoceran community was composed of *Daphnia* spp. (*Daphnia rosea* and *Daphnia pulex*) at every station except at COL, where *Holopedium gibberum* prevailed.

Zooplankton feeding selectivity on algal classes

Although the diatoms dominated the phytoplankton community, specific diatom pigments were quite rare in gut contents (Figs. 1*c*, 1*d*). In contrast, cryptophytes were always dominant, representing between 40% and 100% of the algae detected in the gut contents, except for COL where cryptophyte-derived pigments could be detected neither in POM nor in guts (Fig. 1*c*).

Electivity indices were, for each station, negative for diatoms (Fig. 2). Electivity indices for cryptophytes were posi-

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Table 1. Particulate organic matter (POM) characteristics and POM and zooplankton $\delta^{13}C$ values at the sampling dates.

Station[a]	Sampling depth (m)	Thermocline depth (m)	Chl *a* ($\mu g \cdot L^{-1}$) at DCM	POM C:N	$\delta^{13}C$ (‰) POM at DCM	$\delta^{13}C$ (‰) POM in the epilimnion	$\delta^{13}C$ (‰) Cladocerans
BUL	12	7	2.5	8.7	–34.0	ND	–31.2[b]
COL	13	6	5.5	11.5	–34.2	ND	–29.7[c]
ELL	7	7	29.2	13.5	–29.9	ND	–25.8[b]
GOL	13	7.5	1.7	19.5	–32.5	ND	–31.1[b]
SHL-D	7	6	4.4	18.2	–29.8	–30.6	–30.2[b]
SHL-S	7	7	4.5	18.9	–31.3	–29.6	–23.0[b]
SOL-D	12	10	2.7	15.2	–31.0	–30.1	–28.7[b]
SOL-S	7	10	2.9	15.8	–29.7	–30.5	–29.8[b]

Note: DCM, deep chlorophyll maxima; ND, not determined.

[a]BUL, Butchard Reservoir; COL, Council Lake; ELL, Elk Lake; GOL, Goldstream Reservoir; SHL-D, Shawnigan Lake, deep; SHL-S, Shawnigan Lake, shallow; SOL-D, Sooke Reservoir, deep; SOL-S, Sooke Reservoir, shallow.

[b]*Daphnia* spp.

[c]*Holopedium gibberum*.

Fig. 1. Proportions of diatoms (black), cryptophytes (open), chlorophytes (hatched), and cyanobacteria (grey) in (*a*) particulate organic matter (POM) and (*b*) cladocerans gut contents at the sampled lake stations. BUL, Butchard Reservoir; COL, Council Lake; ELL, Elk Lake; GOL, Goldstream Reservoir; SHL-D, Shawnigan Lake, deep; SHL-S, Shawnigan Lake, shallow; SOL-D, Sooke Reservoir, deep; SOL-S, Sooke Reservoir, shallow.

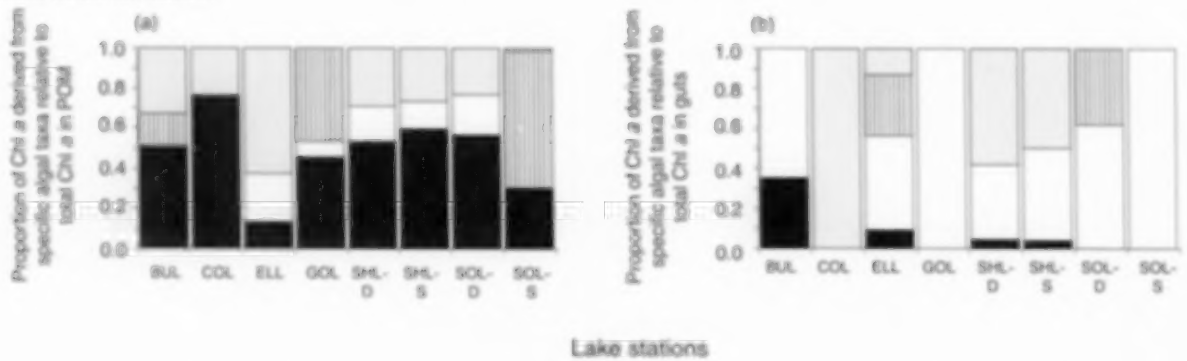
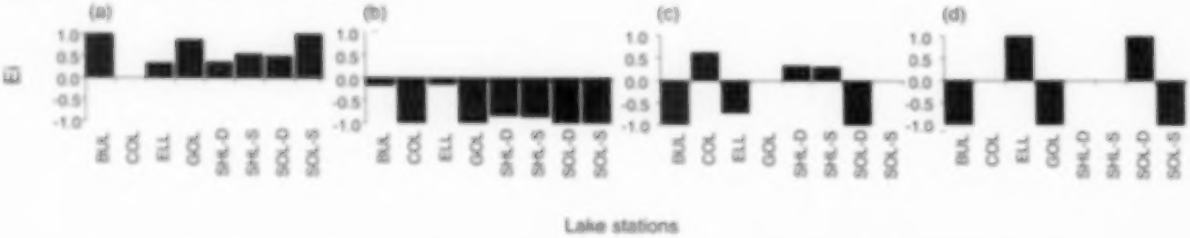


Fig. 2. Values of the electivity indexes (E_i) calculated for (*a*) cryptophytes, (*b*) diatoms, (*c*) chlorophytes, and (*d*) cyanobacteria in filter-feeding cladocerans at the sampled lake stations.



tive at all stations, indicating that filter-feeding cladocerans preferentially ingested cryptophytes. Electivity indices for both chlorophytes and cyanobacteria did not show consistent trends over the stations (Fig. 2).

$\delta^{13}C_{POM}$ values ranged from –34.0‰ to –29.2‰ in July and were thus below the $\delta^{13}C$ value of the terrestrial endmember (Table 1). $\delta^{13}C_{POM}$ sampled from the epilimnion at four stations varied <2‰ from those of POM sampled at the DCM. In addition, $\delta^{13}C$ of epilimnetic POM did not show consistent enrichment in ^{13}C compared with POM sampled at the DCM (Table 1).

$\delta^{13}C_{POM}$ was not correlated with Chl *a* concentrations and C:N ratios (Table 2). However, $\delta^{13}C_{POM}$ was positively correlated with cryptophytes relative biomass and negatively with diatoms relative biomass, since cryptophytes and diatoms contributions to POM were negatively correlated. The linear regression between $\delta^{13}C_{POM}$ and the proportion of cryptophytes in bulk phytoplankton showed that $\delta^{13}C$ in-

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Table 2. Values of the correlation coefficients between $\delta^{13}C_{POM}$ and the predictor variables in July over the set of lakes.

[Chl *a*] ($\mu g \cdot L^{-1}$)	POM C:N	Diatom biomass ($mg \cdot g^{-1}$)	Cryptophyte biomass ($mg \cdot g^{-1}$)	Cyanobacteria biomass ($mg \cdot g^{-1}$)
0.35	0.04	–0.80*	0.97***	–0.49

Note: *, $\alpha = 0.05$; ***, $\alpha = 0.001$.

creased with the contribution of cryptophytes ($\delta^{13}C = -34‰$ when cryptophytes were absent from the phytoplankton community; Fig. 3*a*).

$\Delta\delta^{13}C_{cladocerans}$

$\Delta\delta^{13}C_{cladocerans}$ were positive or close to 0 at all stations (mean $\Delta\delta^{13}C_{cladocerans} = 2.84$, SD = 2.83; Fig. 4). $\Delta\delta^{13}C_{cladocerans}$ was not related to lake trophic status (Kruskal–Wallis test, $P = 0.49$). We found no significant relationships between $\Delta\delta^{13}C_{cladocerans}$ and any of the descriptors used in this study. When $\Delta\delta^{13}C_{cladocerans}$ were calculated from non-lipid-normalized cladoceran $\delta^{13}C$ values, the general pattern of cladoceran ^{13}C enrichment compared with POM was quite robust and did not change substantially (data not shown).

Discussion

Numerous stable isotope studies have been performed to estimate the terrestrial C contribution to aquatic ecosystems production, such as lakes, rivers, and estuaries (Kendall et al. 2001; Bouillon et al. 2003; Carpenter et al. 2005). Using a single phytoplankton end point, authors made the implicit assumption of nonselective zooplankton feeding on algal classes and (or) of a small variability of $\delta^{13}C$ signatures among algal classes compared with the isotopic difference between the two end-members. Results from our study suggest these assumptions are not consistently supported.

Zooplankton feeding selectivity

Previous studies showed that fucoxanthin, lutein, and alloxanthin, respectively diatom-, chlorophyte-, and cryptophyte-specific pigments, could be partially degradated in *Daphnia* guts (Pandolfini et al. 2000). At some stations, amounts of fucoxanthin in cladocerans' gut were lower than that in POM. This could suggest that diatoms, although abundant in the edible size fraction, were poorly ingested at some lake stations. However, numerous studies have reported diatoms' high nutritional quality and pivotal role in fuelling food webs (Brett et al. 2000). Hence, attesting for unambiguous cladocerans' negative selectivity towards diatoms would require more detailed investigations, as part of this pattern might also be attributed to fucoxanthin partial degradation in *Daphnia* guts (Pandolfini et al. 2000) or to the lack of consistent information for the entire growing period.

In spite of possible pigment degradation, amounts of alloxanthin were higher in guts than in POM at all lake stations. Hence, cladocerans exhibited a significant positive selectivity for cryptophytes at all stations. This finding is in line with other field studies reporting *Daphnia*'s positive dietary selection for cryptophytes (Thys et al. 2003). Recently, Hampton et al. (2006) emphasized the dietary key role of

Fig. 3. Relationships between $\delta^{13}C_{POM}$ and the proportion of cryptophytes in phytoplankton.

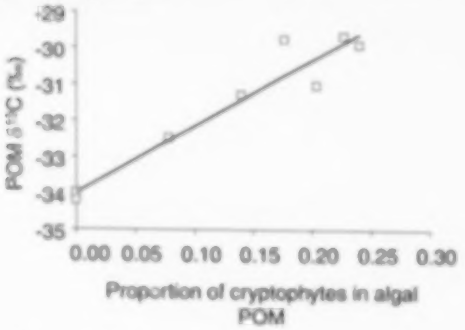
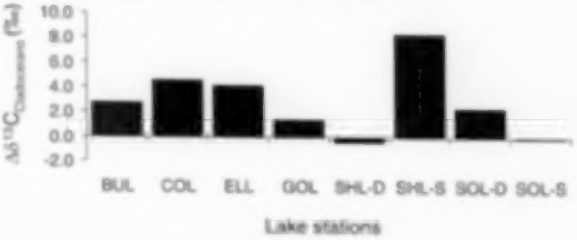


Fig. 4. Values of $\Delta\delta^{13}C_{cladocerans}$, showing the difference between cladocerans and POM $\delta^{13}C$ values at all stations.



cryptophytes in the Lake Washington food web and demonstrated that *Daphnia* could substantially rely on cryptophytes at the detriment of other algal classes. Cladocerans' selectivity for cryptophytes might be passive, as cryptophytes are small and round-shaped algae within the size and shape spectra of easily edible particles for *Daphnia*, or alternatively active, as *Cryptomonas* and *Chroomonas* spp., the major genera of cryptophytes during this summer's sampling at all stations, are high quality food sources (Ahlgren et al. 1992; Ravet et al. 2003). Hence, pigment results provided evidence that cladocerans did not consistently ingest particles within the edible algal size fraction at all stations, as they selected cryptophytes among other algal taxa, thus rejecting the first assumption of nonselective feeding.

Phytoplankton isotopic heterogeneity

Growth is usually considered as a major factor affecting phytoplankton $\delta^{13}C$ (Fry and Wainright 1991; Burkhardt et al. 1999). Increase in specific-growth rate decreases the photosynthetic fractionation rate (Fry and Wainright 1991; Burkhardt et al. 1999). Consequently, higher $\delta^{13}C_{POM}$ values are usually associated with higher primary production or Chl *a* concentrations (Gu et al. 1996). Yet, large $\delta^{13}C$ variability among algal classes would imply species-related differences in the dissolved inorganic C sources used, photosynthetic pathways, and (or) in isotopic fractionation rates (Descolas-gros and Oriol 1992; Leboulanger et al. 1995; Bade et al. 2006) that would have a predominant effect on phytoplankton $\delta^{13}C$.

In the case of $\delta^{13}C$ variability among algal classes much smaller than that between the phytoplankton and allochthonous end-members, phytoplankton $\delta^{13}C$ would be fairly independent from its taxonomic composition and would

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instead be related to primary production. $\delta^{13}C_{POM}$ would, in turn, be essentially dependent on bulk phytoplankton $\delta^{13}C$ (and consequently on primary production) and on the dilution of phytoplankton cells by terrestrial-derived C. In that case, zooplankton, by feeding preferentially on phytoplankton C within POM, would exhibit, at least in oligotrophic and mesotrophic lakes, $\delta^{13}C$ values lower than that of POM (Del Giorgio and France 1996; Grey et al. 2000). As every algal class would exhibit fairly similar $\delta^{13}C$ values, it would be expected that even selective feeding of zooplankton does not strongly affect their $\delta^{13}C$ signatures. In contrast, if $\delta^{13}C$ values are substantially different among algal classes, both phytoplankton and $\delta^{13}C_{POM}$ would essentially depend on the taxonomic composition of phytoplankton rather than on primary production. The difference between zooplankton and POM $\delta^{13}C$ would then depend on zooplankton feeding selectivity on different algal classes.

In our lake study, $\delta^{13}C_{POM}$ was closely related to the phytoplankton taxonomic composition, especially to the relative proportion of cryptophytes versus diatoms. In lakes where cryptophytes were absent, POM had consistent $\delta^{13}C$ values (–34‰). POM was ^{13}C-enriched as the proportion of cryptophytes increased. Cryptophytes represented up to 25% of the phytoplankton biomass in ELL, SHL-D, and SOL-S, where $\delta^{13}C_{POM}$ values reached –30‰. This pattern would be consistent with cryptophytes being enriched in ^{13}C compared with the rest of the phytoplankton community. In addition, $\delta^{13}C$ values of cladocerans were consistently higher than those of POM in every lake in July, implying that cladocerans consistently fed on a ^{13}C-rich food source. Previous lake studies showed depth-related differences of $\delta^{13}C_{POM}$ values with higher values in the mixed layer and lower values in the hypolimnion as a consequence of the vertical stratification of photosynthesis and respiration processes (Del Giorgio and France 1996). However, in our set of lakes, $\delta^{13}C_{POM}$ in the epilimnion did not show consistent enrichment compared with the $\delta^{13}C$ values of POM at the DCM. These data suggest that ^{13}C enrichment of cladocerans relative to POM are related to preferential feeding on the ^{13}C-enriched cryptophytes rather than grazing on epilimnetic POM.

Cladocerans $\delta^{13}C$ were as high as –23‰ and –25‰ in ELL and SHL-S, respectively (i.e. even higher than that of the terrestrial $\delta^{13}C$ (–27.5 ‰ in these watersheds; Perga et al. 2006). Gut pigment analysis on these lakes revealed that although cryptophytes were positively selected by the cladocerans, diatoms and cyanobacteria were also ingested. These results suggest, therefore, that cryptophytes could reach $\delta^{13}C$ values heavier than –23‰ for this summer's sampling. Therefore, we argue that cryptophytes could be consistently ^{13}C-enriched compared with these diatoms whose biomass dominated at this time, but also ^{13}C-enriched compared with the terrestrial end-member.

Our study indicates there was a strong isotopic heterogeneity within coexisting algal taxa at a single sampling period in these study lakes, although these conclusions were reached through indirect evidence via pigment analysis. We argue, however, that cryptophyte ^{13}C enrichment relative to the rest of the phytoplankton community might have been difficult, if at all possible, to reveal from direct evidence. Taxon-specific stable isotope signatures of lake phytoplankton have mostly been measured in mesotrophic or eutrophic lakes (Zohary et al. 1994; Vuorio et al. 2006). Specific stable isotope signatures were measured essentially during bloom periods with low phytoplankton diversity. Isolation of phytoplankton taxa for isotope analyses is strongly depending on their dominance in the phytoplankton community. In our study, cryptophytes biomass represented <25% of the whole community biomass in the <35 µm size class in lakes that are mostly oligotrophic, which prevented us from isolating enough biomass to run species-specific isotope analyses. Using cell sorting by cytometry coupled with a gas chromatograph – isotope ratio mass spectrometer (GC-IRMS) (Pel et al. 2003) may provide direct evidence of cryptophytes ^{13}C enrichment compared with the rest of the algal community, provided that enough cryptophyte material could be isolated from POM by cell cytometry sorting methods. However, this equipment is not readily available and the cost for analyses is quite high.

Studies on species-specific isotope composition of phytoplankton are scarce, but they provide evidence of large isotopic variability among coexisting phytoplankton taxa (Zohary et al. 1994; Pel et al. 2003; Vuorio et al. 2006). Pel et al. (2003) measured significantly lower $\delta^{13}C$ values in prochlorophytes than in other phytoplankton (essentially represented by diatoms), while cyanobacteria were generally enriched in ^{13}C compared with diatoms and green algae (Vuorio et al. 2006). The dinoflagellate *Periodiniosis* had significantly higher $\delta^{13}C$ values than the rest of phytoplankton in Lake Kinneret (Zohary et al. 1994), such as the chlorophyte *Volvox* sp. and the dinoflagellate *Ceratium hirundella* in Lake Suwa (Yoshioka et al. 1994). High $\delta^{13}C$ values for cryptophytes have also been observed in oligotrophic UK lakes (J. Grey, Queen Mary, University of London, School of Biological Sciences, London E1 4NS, UK, personal communication), but cryptophyte $\delta^{13}C$ signatures have been, to our knowledge, only poorly investigated. However, such a variability in the isotopic composition of phytoplankton taxa both within coexisting species in a single lake at a single sampling date or even between lakes is still difficult to interpret and cannot be easily linked to their physicochemical environment (Vuorio et al. 2006). Our data set may not allow us to address the causes of such an isotopic heterogeneity within phytoplankton.

Consequences on estimates of allochthonous C contribution to zooplankton secondary production

There was an overlap between the range of $\delta^{13}C$ values of the different algal classes within the phytoplankton community and those values of bulk phytoplankton and allochthonous end-members. In addition, zooplankton selectively ingested ^{13}C-enriched cryptophytes at the detriment of ^{13}C-depleted diatoms. In that context, the actual phytoplanktonic food source of cladocerans was substantially ^{13}C-enriched compared with bulk phytoplankton. Hence, none of the prespecified assumptions were fulfilled. To evaluate how these results may affect estimates of zooplankton allochthony, we compared outputs from a classical two-sources mixing model (model 1) to those from a model that accounts for phytoplankton isotopic heterogeneity (model 2). COL was excluded from the data set, as cryptophytes were not detected in POM or in cladocerans gut contents.

To account for variations of phytoplankton $\delta^{13}C$ in both

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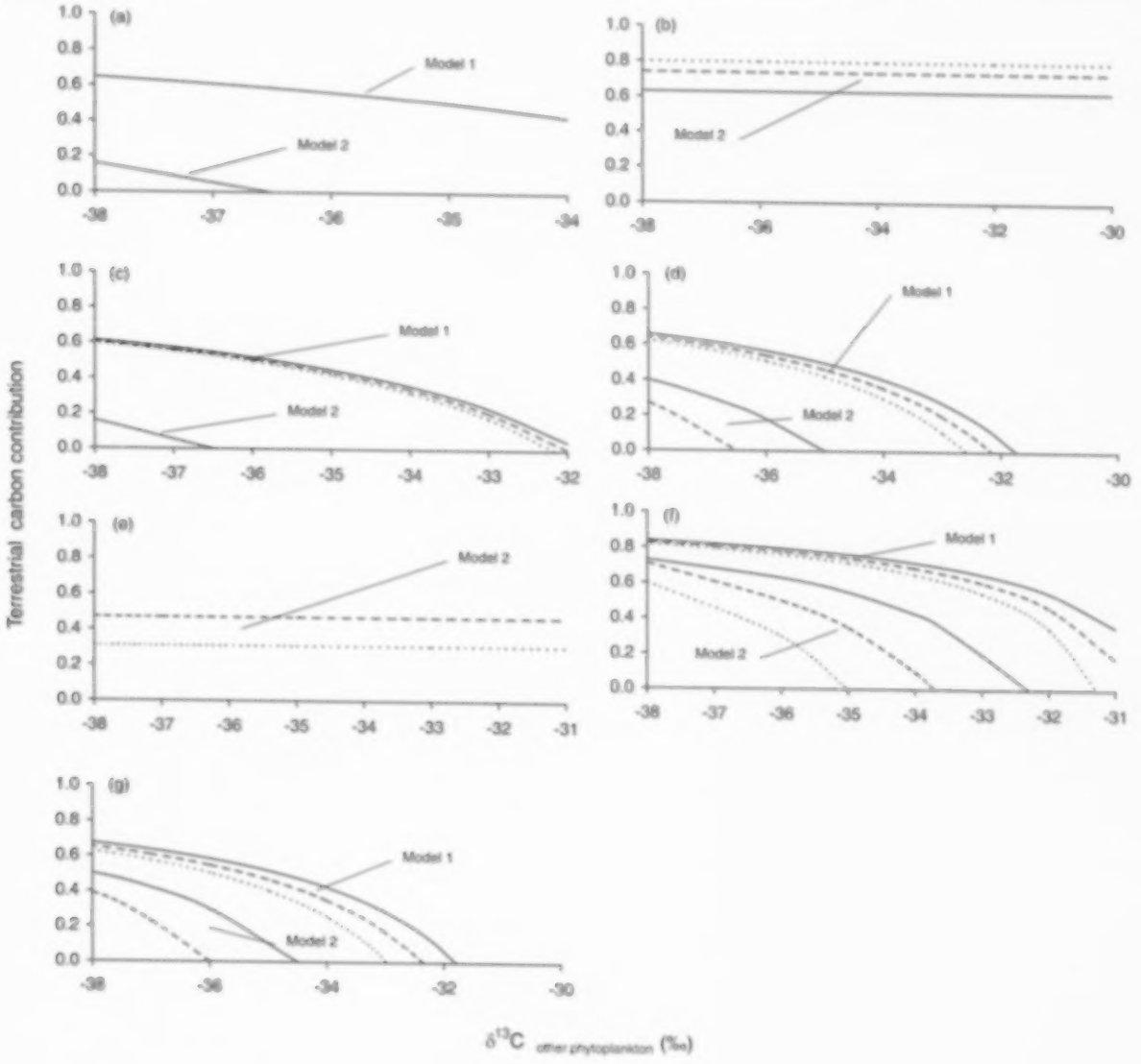
Fig. 5. Estimates for terrestrial C contribution to *Daphnia* secondary production, computed from model 1 (two-source mixing model) and model 2 (three-source mixing model), for $\delta^{13}C$ values of cryptophytes ($\delta^{13}C_{cryptophytes}$) of –23‰ (continuous line), –21‰ (large dashed line), and –19‰ (small dashed line) and $\delta^{13}C$ values of the other phytoplankton taxa ($\delta^{13}C_{other\ phytoplankton}$) at the considered lake station: (*a*) Butchard Reservoir, (*b*) Elk Lake, (*c*) Goldstream Reservoir, (*d*) Shawnigan Lake, deep, (*e*) Shawnigan Lake, shallow, (*f*) Sooke Reservoir, deep, and (*g*) Sooke Reservoir, shallow. For model 2, results were reported as maximal terrestrial C contribution to *Daphnia* secondary production.



models, we considered that $\delta^{13}C$ values for cryptophytes and for the other algal taxa may vary over large isotopic ranges. For cryptophytes, we considered cases where their $\delta^{13}C$ values could range between –23‰ and –19‰ at all lake stations; for the other taxa, we allowed their isotopic composition to range between $\delta^{13}C_{POM}$ values from each lake station and –38‰.

In model 1, bulk phytoplankton $\delta^{13}C$ values ($\delta^{13}C_{phytoplankton}$) were computed using the following mixing model:

$$(1)\quad \delta^{13}C_{phytoplankton} = \alpha_{cryptophytes}.\delta^{13}C_{cryptophytes} + (1 - \alpha_{cryptophytes}).\delta^{13}C_{other\ phytoplankton}$$

with $\alpha_{cryptophytes}$ (percent contribution of cryptophytes within phytoplankton) estimated from pigments measures in POM; $\delta^{13}C_{cryptophytes}$ is isotope composition of cryptophytes, and $\delta^{13}C_{other\ phytoplankton}$ is isotope composition of other algal classes. Allochthonous C contribution to cladocerans ($\alpha_{terrestrial}$) was then assessed by a classical two-sources mixing

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model from the isotope composition of the terrestrial end-member ($\delta^{13}C_{terrestrial}$ = –27.5 ‰) and $\delta^{13}C_{phytoplankton}$ computed in eq. 1.

$$(2)\quad \delta^{13}C_{cladocerans} = \alpha_{terrestrial}\cdot\delta^{13}C_{terrestrial} + (1-\alpha_{terrestrial})\cdot\delta^{13}C_{phytoplankton}$$

For model 2, Isosource (www.epa.gov/wed/pages/models/stableIsotopes/isosource/isosource.htm; Phillips and Gregg 2003) was used to compute a range of estimates for the contribution of the three end-members considered as distinct C sources (cryptophytes, other phytoplankton taxa, and terrestrial C) to cladoceran secondary production. However, as inputs of $\delta^{13}C$ values for cryptophytes and other algae were already large, additional constraints were used to restrict the range of output estimates. Consistent with pigment results, only outputs with cryptophyte contribution to the total phytoplankton resource >40% were considered (Fig. 5). As this probabilistic model provides a range of potential contributions for each source, results were reported as maximal terrestrial C contribution to *Daphnia* secondary production. Hence, to evaluate how phytoplankton isotopic heterogeneity may affect estimates of zooplankton allochthony, terrestrial C contribution to *Daphnia* secondary production assessed from model 1 was compared with maximal terrestrial C contribution estimated from model 2.

Very high $\delta^{13}C$ values of cladocerans in SHL-S and ELL ($\delta^{13}C_{cladocerans} > \delta^{13}C_{terrestrial}$) could not be explained when relying on a simple two-source mixing model, and no estimates for allochthonous C contribution could be computed. For the other lake stations, outputs from the two-source mixing model were always higher than those from the three-source model.

Thus, high $\delta^{13}C$ values in cladocerans were not directly linked to terrestrial-derived C, which is generally enriched in ^{13}C compared with bulk plankton, but to preferential feeding on specific algal taxa that were enriched in ^{13}C compared with the rest of the phytoplankton community. As a consequence, applying $\delta^{13}C$ signatures of bulk phytoplankton in a two-source mixing model, as was commonly done in previous studies, overestimated at most lake stations the contribution of terrestrial C to zooplankton secondary production.

Based on classical two-sources mixing models, previous lake studies of natural abundance stable isotopes usually found high contributions of terrestrial C to lake food webs (Karlsson et al. 2003; Perga et al. 2006). However, such studies are based on analytical methods that do not consider possible different algal stable C isotopic signatures. Our results draw clear attention to phytoplankton isotopic heterogeneity, as $\delta^{13}C$ variations of specific ingested algae are likely to mislead estimations of the relative contribution of terrestrial C to zooplankton secondary production. New analytical tools need to be developed and made accessible to deal directly with phytoplankton isotopic heterogeneity. Cell cytometry coupled with GC-IRMS might be one (Pel et al. 2003; Pace et al. 2007). An alternative way to estimate terrestrial C subsidies to lake food webs relied on ^{13}C-enriched dissolved inorganic carbon (DIC) addition, resulting in artificially high $\delta^{13}C$ values for phytoplankton (Carpenter et al. 2005; Pace et al. 2007). In such studies, the two endpoint sources (terrestrial and autochthonous organic matter) had so highly different $\delta^{13}C$ values that the impact of $\delta^{13}C$ variability between algal species might have been substantially lowered. However, in such studies, ^{13}C-enriched DIC was added in the mixed layer of the water column, thereby marking phytoplankton species actually growing within these depths. If, in these lakes, as in our set of lakes in July, the algae that substantially support zooplankton secondary production were actually grown below the thermocline, they would not have access to the ^{13}C-enriched DIC and would therefore not be labelled with ^{13}C. In that context, terrestrial C contribution to the lake food web could also have been overestimated.

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Reconstructing historical stock development of Atlantic cod (*Gadus morhua*) in the eastern Baltic Sea before the beginning of intensive exploitation

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Abstract: The landings of Atlantic cod (*Gadus morhua*) in the eastern Baltic Sea in the early decades of the 20th century were below 50 thousand tonnes and therefore lower than in recent years at very low stock size. These low landings have largely contributed to a perception that the stock size was also low before the 1950s. In this investigation, we demonstrate that cod spawning stock biomass in the years 1925–1944 fluctuated in a similar range as in the periods from the 1950s to the mid-1970s and from the late 1980s onwards and was in most of these years at least twice as high as at present. Fishing mortality before the 1940s was below 0.2, but reached moderate levels during the Second World War. The stock size before the war may be considered as a reference level of biomass at low fishing impact, providing important information for the management of fisheries and the Baltic ecosystem.

Résumé : Les débarquements de morues (*Gadus morhua*) de l'est de la Baltique des premières décennies du 20e siècle étaient inférieurs à 50 mille tonnes et donc plus faibles que ceux des années récentes caractérisées par une taille du stock très réduite. Ces faibles débarquements ont en grande partie contribué à la perception que la taille du stock aussi était basse avant les années 1950. Dans notre travail, nous démontrons que la biomasse du stock reproducteur de la morue dans les années 1925–1944 fluctuait dans la même gamme de valeurs que durant les périodes des années 1950 au milieu des années 1970 et des années qui ont suivi la fin des années 1980; le stock était pour la plupart de ces années au moins le double du stock actuel. La mortalité due à la pêche avant les années 1940 était inférieure à 0,2, mais elle a atteint des valeurs moyennes au cours de la Seconde Guerre mondiale. La taille du stock avant la guerre peut ainsi représenter une valeur de biomasse de référence lorsque l'impact de la pêche était faible, ce qui fournit des données importantes pour la gestion des pêches et de l'écosystème de la Baltique.

[Traduit par la Rédaction]

Introduction

Most of the regular fish stock assessments in the North Atlantic Ocean cover only the recent three–four decades, and the available time series of stock developments begin after the onset of intensive exploitation. Consequently, the baselines for rebuilding depleted fish stocks may only refer to contemporary developments (Pitcher 2001), whereas a longer term historical perspective is important for evaluating the state of marine ecosystems and setting achievable goals for their management (Jackson et al. 2001; Pinnegar and Engelhard 2008). Several major shifts that have occurred in the abundances of fish stocks over decades or centuries have been related to exploitation. For example, studies on historical developments of Atlantic cod (*Gadus morhua*) stocks near Newfoundland (Rose 2004) and on the Scotian Shelf, eastern Canada (Rosenberg et al. 2005) showed that fishing was the dominant factor responsible for century-scale declines of these populations. Jackson et al. (2001) have also concluded that stock abundances of several predator species at the time of lower fishing pressure were much higher compared with the present low levels.

In the Baltic Sea, the low landings in the first decades of the 20th century suggest the opposite. The annual cod landings before the 1940s were below 50 thousand tonnes, which is not only lower than during the more recent decades, but is low also when compared with landings in neighbouring areas. Before the 1940s, the cod landings, for example, in the North Sea were up to 100 times higher than that in the Baltic Sea, whereas the landings from the two areas in the later decades have been at a relatively similar level (Fig. 1). However, the low Baltic cod landings in the early decades of the 20th century may not reflect the stock size but could also be due to an undeveloped fishery or low local demand for cod (MacKenzie et al. 2002; Bager et al. 2007; Eero et al. 2007*b*).

Biomass estimates for the eastern Baltic cod are available from International Council for the Exploration of the Sea (ICES) assessments from 1966 onwards (e.g., ICES 2007), and the time series has recently been extended back to 1946 (Eero et al. 2007*a*). Throughout this period, the stock has been relatively heavily exploited. The stock size and the exploitation rate earlier in the 20th century are at present poorly known, as the available estimates (Thurow 1999) are uncertain. The fishing intensity before the 1950s is considered to have been lower than that in later decades (Bagge et al. 1994). However, even at relatively low effort, fishing

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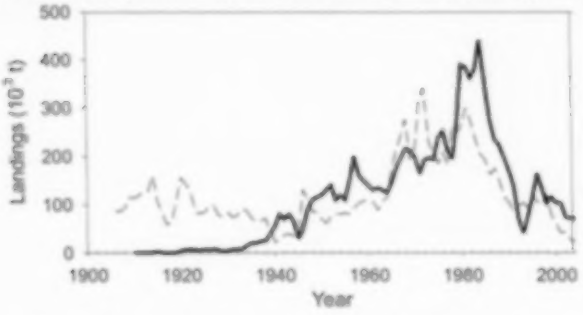
Fig. 1. Cod (*Gadus morhua*) landings in the Baltic Sea (Sparholt 1994; ICES 2007) compared with the North Sea (Daan et al. 1994; ICES 2005), represented by solid and broken lines, respectively.



could still have had a major impact on the stock if the biomass was as low as suggested by Thurow (1999). The apparently low biomass of fish stocks in the early decades of the 20th century has been considered to be related to low productivity of the Baltic Sea before the 1950s (Thurow 1997*a*).

The uncertainty in the cod biomass before the mid-1940s is related to scarcity of data for estimating the stock size by standard methods with satisfactory reliability. In this paper, we compile the available historical evidence that could be utilized to provide an indication of the cod stock size in the period from 1925 to 1944. We use multiple approaches based on independent data sets that enable us to evaluate the consistency of the information provided by the different analyses. The estimated biomass and fishing mortality allow us to evaluate whether the stock size in these years can be used as a baseline before the onset of heavy fishing pressure. Our analyses contribute to improving the understanding of the relative importance of environmental and human factors on development of the cod stock and provide new knowledge for management of fisheries and the Baltic ecosystem.

Materials and methods

We used four approaches to estimate the stock size of eastern Baltic cod (ICES subdivisions (SDs) 25–32, Fig. 2) in 1925–1944. First, spawning stock biomass (SSB) was estimated based on total mortalities obtained from catch curve analyses. This included analyses of length distribution data, compilation of catch numbers at length, and subsequent estimation of mortality and biomass. Second, an indication of the level of SSB was obtained by comparing the catch rates of RV *Poseidon* in the period from 1925 to 1938 with the data from RV *Solea* for the years 1975–1992. Third, we analysed cod landings in the northernmost areas in the Baltic Sea and in the Gulf of Riga where cod abundance is more variable and dependent on stock size. Fourth, we compared egg abundances from ichthyoplankton surveys in the 1920s and 1930s with more contemporary data. All the utilized data refer to the eastern Baltic stock (SDs 25–32). All four methodologies used here, as do all methods for quantifying population sizes of wild organisms, involve different assumptions and are associated with different levels of uncertainty, partly resulting from incomplete data coverage in

Fig. 2. International Council for the Exploration of the Sea (ICES) subdivisions in the Baltic Sea.

time and space. The major uncertainties related to these approaches are addressed in the Discussion. The data sources and treatments concerning each of the analyses are described below.

Length distribution data

Length distributions of cod in the catches by German cutters and steamers for the period from 1940 to 1944 and by the former USSR, Poland, Federal Republic of Germany, and former German Democratic Republic for the period from 1947 to 1975 (Table 1) were included in estimating mortalities in respective time periods. For the period before the 1940s, average length distributions were additionally presented for German trawl surveys conducted in the period from 1925 to 1938 between March and July by RV *Poseidon* using commercial trawl (Kändler 1944). For comparison, average length frequency was presented for surveys by RV *Anton Dohrn* in the years 1962–1970 during April–May (Tiews 1974). To compare commercial length distributions in the 1940s with later decades, we have also included average length structure of Danish commercial cod landings in the years 2002–2006 obtained from the national database. Differences in the proportions of cod ≥ 50 cm in length in the catches in different time periods were analysed using Wilcoxon rank test.

Calculation of catch numbers by length groups

1940–1944

The total international eastern Baltic cod landings were estimated at 52.5, 69.9, 53.7, 71.9, and 51.6 thousand tonnes in 1940–1944, respectively. Approximately 80% of the landings were taken by German steam trawlers. The recorded landings of steamers included all species; however, ~99%

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Table 1. Availability of length composition data for the landings by German cutters and steamers in 1940–1944 and for the landings by the former USSR, Poland, former German Democratic Republic (GDR), and Federal Republic of Germany (FRG) in 1947–1975 by year, time, and subdivision (SD) and respective data sources.

Country	Year	Time	SD	Source
Germany (cutters)	1940	May, June	25	Meyer 1952*b*
	1941	Feb.	26	
	1943	Mar., Nov.	26	
	1944	Jan.	26	
Germany (steamers)	1940	May–July	25, 26, 28	Meyer 1952*b*
	1941	Mar., Feb., Dec.	25, 26, 28	
	1942	Jan., Apr.–June	25, 26	
	1943	Mar., July, Sept.	25, 26, 28	
	1944	Feb., Apr., June	26, 28	
USSR	1947–1956	Spawning time	26–32	Dementjeva 1959
	1966–1975	Annual mean	26–32	Presented to ICES
Poland	1947	Annual mean	26	Chrzan 1950
	1948–1951	Annual mean	26	Chrzan 1954
	1952–1954*	Annual mean	26	Chrzan 1957
	1955–1956*	Annual mean	26	Rutkowicz 1959
	1953–1954	Annual mean	25	Reimann 1962
	1955–1956	Annual mean	25	Stanek 1962
	1957–1960	Annual mean	25	Stanek 1965
	1966–1975	Quarter 1	25, 26	Kosior 1967, 1969, 1970, 1971, 1972, 1973, 1974, 1976
GDR	1954–1960	Quarters 1 and 2	25	M. Berner, unpublished
	1967–1975	Quarters 1 and 2	25	Berner and Borrmann 1980; M. Berner, unpublished
FRG	1969–1975	Quarters 1 and 2	25–28	Presented to ICES

*Length distributions in weight were converted into numbers of fish applying mean weight-at-length in 1947–1954 (Chrzan 1962).

was reported to have been cod (Meyer 1952*b*). The German steamer fleet operated in offshore areas in the Baltic Sea, mainly in Bornholm Basin, Gdansk Deep, and Gotland Basin (Meyer 1952*a*), which correspond to the spawning areas of the eastern Baltic cod (Bagge et al. 1994). The major part of the German cod fishery in the Baltic in 1940–1944 took place in spring (March–June), targeting spawning aggregations (Meyer 1952*a*). The fishing effort of steam trawlers during the years from 1940 to 1944 was relatively stable; number of fishing trips in the Baltic in these years varied between 1011 and 1177 without a temporal trend, whereas the number of fishing days per trip increased from 9.9 to 11.7 during 1940–1944 (Meyer 1952*a*).

Danish landings in the years 1940–1944 were included for the harbours located at Christiansø and at the east coast of Bornholm Island, assuming that the cod landed in these ports was caught in the waters east of Bornholm, i.e., in SDs 25–32. The data were extracted from the statistics of the Danish Directorate of Fisheries stored in the Danish National Archive. Swedish landings were also included for the counties bordering SDs 25–32 (Awebro and Poulsen 2002).

Length compositions from German cutters (Meyer 1952*b*) were assumed to represent the part of cod landings not taken by German steamers in 1940–1944. The average length composition in the years 1941 and 1943 was applied for 1942. The data for different months in 1940 and in 1943 were averaged to obtain annual means. The length compositions of the steamer catches by month were averaged by using respective landings as weights (Meyer 1952*a*). Mean weights-at-length from Polish data for the years 1947–1954 (Chrzan 1962) were used to calculate landings in numbers by length groups in 1940–1944.

1947–1975

Catch numbers-at-length were calculated for the years 1953–1960 for SD 25, for the years 1947–1956 for SDs 26 and 28, and for the years 1966–1975 for SDs 25, 26, and 28. Whenever length distributions from several countries for a given year and area were available, these were averaged, weighting by respective landings. Landings data by countries and SDs before 1972 were compiled by Eero et al. (2007*a*); from 1972 on, the data are available in ICES (e.g., ICES 2007). Average weights-at-length in 1953–1954 (Reimann 1962) and 1954–1962 (M. Berner, Institute for Baltic Sea Fisheries, Alter Hafen Süd 2, 18069 Rostock, Germany, unpublished data) and the average weights in 1967–1975 (M. Berner, unpublished data) were applied in calculating catch numbers-at-length for SD 25 in 1953–1960 and 1966–1975, respectively. The catch numbers in SDs 26 and 28 in 1947–1956 and 1966–1975 were calculated using mean weights-at-length for the years 1947–1954 (Chrzan 1962) and 1960–1975 (data from the former USSR, presented to ICES Working Group on Demersal Fish Stocks in the Baltic, available from Latvian Fish Resources Agency, Daugavgrivas Str. 8, LV-1048 Riga, Latvia), respectively.

Estimation of mortality and biomass based on catch curve analyses

The length-converted catch curve method (Sparre and Venema 1998) was used to calculate total mortalities. Before the mid-1940s, mortalities were estimated based on average catch numbers-at-length in 1940–1941, in 1942–1944, and for comparison in 1940–1944 combined. The two time periods were kept separate because of large differences

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Table 2. Availability of catch-per-unit-effort (CPUE) data from German research surveys in Bornholm Basin by vessel and year and characteristics of the vessels and fishing gear.

Vessel	Length (m)	Capacity (BRT[a])	Engine power (hp[b])	Gear[c]	Year	No. of stations	Month
Poseidon	46	453	480	120-foot trawl	1925–1926	11	Apr., July
					1929, 1931	21	Apr.–June
					1933, 1935	22	Mar.–Apr.
					1938	19	Mar., Aug.
Anton Dohrn	62	999	850	180-foot standard herring trawl and 140-foot fish trawl with herring cod end	1964	13	Apr.–May
					1966	14	Apr.
					1968	11	Apr.
					1969	23	Apr.
					1970	13	Apr.–May
					1971	12	Apr.–May
Solea	35	337	870	Sonderburg trawl	1975–1992	~25 per year	Mar.–May
				Cod hopper; 107-foot trawl[d]	Annual		

[a]BRT, brutto register tonnage.

[b]1 hp = 746 W.

[c]The measure of trawl size refers to the groundrope length in feet (1 foot = 0.3048 m).

[d]The length of the groundrope was not provided as an official technical measure of the trawl; the respective measurement was obtained from the design plan to compare with the measures of the trawls used by *Poseidon* and *Anton Dohrn* (M. Stein, Sea Fisheries Institute, Palmaille 9, D-22767, Hamburg, Germany, personal communication).

in length frequency. For comparison, we estimated mortalities based on the length data also for the period from 1947 to 1975, as for these years the estimates from age-based virtual population analysis (VPA) were available for validation (Eero et al. 2007*a*).

The length-converted catch curve method uses the von Bertalanffy growth equation to convert length into age. Three sets of growth parameters estimated for the 1960s and 1970s for SD 25 (Kosior 1976; Berner and Borrmann 1980; Steffensen and Bagge 1983) and for SD 26 (Draganik and Netzel 1966; Lablaika et al. 1975; Kosior 1976) were applied in estimating mortalities in SD 25 and in SDs 26 and 28, respectively. The mortalities for the years 1940–1944 were estimated for all six sets of growth parameter values. Different values were applied to evaluate the sensitivity of mortality estimates to growth parameters. Length groups from 45 to 70 cm were included in catch curves, which corresponded approximately to ages 4–7 after conversion. The larger fish were not included because of low numbers in the samples and because of increasing uncertainty in the relationship between age and length for larger fish (Sparre and Venema 1998).

To have an indication of possible differences in growth in the 1940s compared with the later years, we used the information on condition of cod. Fulton's condition factors (K) for cod in SD 25 in the first quarter of 1943 were estimated by Meyer (1952*c*). His calculations were based on weights of the fillets multiplied by two, as the fillets formed approximately half of the total weight of the fish. As the exact proportions of the fillets in the total weight were also given (between 50% and 60%; Meyer 1952*c*), we used these data to adjust Meyer's estimates of condition factors for more accurate values. The average condition factor of cod in SD 25 in the first quarter of the years 1991–2007 was calculated based on length and total weight data from the Baltic International Trawl Surveys (BITS, ICES database: www.ices.dk/datacentre/datras/public.asp).

Our catch curve analyses were based on average catch-at-length over a number of years, not following the development of single year classes. To evaluate the consequent possible bias in mortality estimates for 1947–1975, we additionally estimated mortalities in this period from age-based catch curves. Catch numbers-at-age (Eero et al. 2007*a*) were averaged over the same time periods as the length-based data. Age groups 4–7 were included in catch curves. The mortality estimates from catch curves were then compared with the results from VPA (Eero et al. 2007*a*).

Fishing mortalities in 1940–1941 and 1942–1944 were calculated from respective total mortality values, assuming a natural mortality rate of 0.2 that corresponds to the value applied in the assessment of this stock (ICES 2007). The biomass was estimated as a ratio between the landings and the fraction of the stock removed by the fishery, the latter being represented by fishing mortality calculated as a percentage. The estimated biomass was considered to represent the size of the spawning stock, as the mortality estimates did not include small fish. This was supported by the data for the years 1966–2006, where the ratio between the landings and the fraction removed due to fishing mortality of the ages 4–7 produced values similar to the estimates of SSB ($r^2 = 0.979$, $p < 0.01$; ICES 2007).

Catch per unit of effort

Catch rates (kg·h^{-1}) of RV *Poseidon* (Table 2) in Bornholm Basin (SD 25) in the period from 1925 to 1938 (Kändler 1944) were compared with catch rates by RV *Anton Dohrn* in the years 1964–1971 (Tiews 1974; K. Tiews, Institute of Sea Fisheries, Palmaille 9, 22767 Hamburg, Germany, unpublished data) and from RV *Solea* for the years 1975–1992 (Thurow and Weber 1992).

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The catch rates (kg·h^{-1}) by RVs *Anton Dohrn* and *Solea* in SD 25 were both significantly correlated with cod SSB in SDs 25–32 in respective years ($r^2 = 0.673$, $p < 0.05$; $r^2 = 0.441$, $p < 0.01$, respectively). The average catchability calculated as a ratio between CPUE (kg·h^{-1}) and SSB (thousand tonnes) was 0.66 (±0.21) for RV *Solea* and 1.2 (±0.45) for RV *Anton Dohrn*. The technical characteristics of the gear in use and the vessels (Table 2) indicated that catchability of RV *Poseidon* was closer to RV *Solea* than to RV *Anton Dohrn*. Therefore, the average ratio between CPUE and SSB from RV *Solea* was applied for calculating SSB in the 1920s and 1930s based on the catch rates of RV *Poseidon*.

Cod landings in the northern Baltic Sea and in the Gulf of Riga

Increased cod landings in the northern Baltic Sea and in the gulfs can be used as an indicator of increased distribution area and stock abundance (Ojaveer et al. 1985; Ojaveer et al. 1999; MacKenzie et al. 2002). Therefore, we compared cod landings in the Gulf of Riga and in SDs 29–32 in the period from the mid-1920s until the early 1940s with the landings in these areas after the 1970s. Similar landings in the 1920s–1940s compared with the landings in the later period were assumed to indicate similar levels of SSB.

The development of landings in the Gulf of Riga was represented by Latvian data extracted from national fisheries statistical reports. In the years 1925–1938, the landings reported for the harbours located along the Gulf of Riga were assumed to have been taken from the Gulf. For SDs 29–32, two time series of landings were analysed: (*i*) Swedish landings in SDs 29–31; and (*ii*) Estonian and the former USSR landings in SDs 29 and 32. The Estonian statistics refers to the years 1934–1938 and 1991–2004; in 1973–1990 the landings reported by the former USSR in SDs 29 and 32 were utilized. Swedish data until the year 1974 were from national fisheries statistics (Awebro and Poulsen 2002) and included landings for the counties corresponding to the location of SDs 29–31. Estonian landings by counties in 1934–1938 were extracted from the national fisheries statistical reports and included the areas located at the west and north coast corresponding to SDs 29 and 32. The Swedish and Estonian (former USSR) data from 1975 and 1973 onwards, respectively, were available from ICES (2007).

Our comparisons of landings in the northern Baltic and in the Gulf of Riga assumed that the proportions of the stock dwelling in these areas were similar in the time periods compared. The expansion of the cod distribution northeastwards at higher population sizes is related to increased salinities that favour cod reproduction (Lablaika and Lishev 1961; Plikshs et al. 1993; Köster et al. 2005). To account for possible differences in stock distribution due to different salinities, we compared only the landings from time periods with similar salinity conditions in the bottom water. The landings from the mid-1980s onwards were therefore only used in comparisons with the landings in the early 1930s, when the salinities were similarly low (Fonselius 1962; Köster et al. 2005). The landings in the mid-1920s and late 1930s were compared with the data for the 1970s.

Egg abundance

Potential egg production of eastern Baltic cod stock taking into account maturity, sex ratio, and relative fecundity has been shown to be highly correlated with realized egg production from ichthyoplankton surveys in SD 25 (Kraus et al. 2002). Based on this information, a recent attempt to estimate SSB from egg abundance for SD 25 (G. Kraus, H.-H. Hinrichsen, R. Voss, J. Tomkiewicz, E. Teschner, M. Schaber, F.W. Köster, unpublished data) indicated that the method is sensitive to the hydrographic conditions with a good agreement in inflow years, but less agreement in stagnation periods. As sufficient information for the period before the 1940s is not available for utilizing the relationship between potential and realized egg production for estimating SSB, we followed a simpler approach to obtain a proxy for the level of SSB from available egg abundances for the 1920s and 1930s. We compared egg abundances in SD 25 in 1926, 1931, and 1938 (Mielck and Künne 1935; Kändler 1944) with data from the same area from 1966 onwards (Köster et al. 2005). To account for possible differences in buoyancy of eggs at different hydrographic conditions (Wieland and Jarre-Teichmann 1997), egg abundances were compared only for years where the bottom water salinities (80 m depth; Mielck and Künne 1935; Kändler 1944; Fonselius and Valderrama 2003; ICES oceanographic database) were similar. Similar egg abundances observed at similar salinities were assumed to reflect similar level of SSB.

The egg data for 1926, 1931, and 1938 were from July, May–June, and July–August, respectively. The data from 1966 onwards represented peak in spawning time, i.e., April–June up to 1989 successively shifted to mid-April to July (1990), May–July (1991–1992), mid-May to mid-August (1993–1995), and mid-June to mid-September (1996 to 1999) corresponding to the shift in spawning time (Köster et al. 2005). In 1926, 1931, and 1938, data were available from 7, 8, and 40 stations, respectively. For the later years, data were generally from three surveys per year, with the number of stations ranging from 24 to 46 per survey (CORE 1998).

Results

Changes in length composition of the landings and condition of cod

In the years 1940–1941, more than 60% of cod in the landings by German steamers were ≥50 cm in length. Thereafter, the proportion of large fish declined, and in 1942–1944 they formed as an average only 49% of the landings (Fig. 3), with the difference in the proportion being significant ($W = 69$; $p < 0.05$). An even larger reduction in the percentage of cod ≥ 50 cm was indicated in the landings of cutters. The proportion of fish at this size in the landings in 1938–1941 was on average more than twice as high as that during 1942–1944. However, the difference was not found significant likely because of a low number of samples.

The average length distribution of cutter catches in the years 1942–1944 was similar to the average commercial catch compositions in the later time periods, i.e., 1947–1960, 1966–1975, and 2002–2006 (Fig. 3). The average proportion of cod ≥ 50 cm in these years was between 16% and 19%. Similar length structures were observed in research catches in the period from 1962 to 1970, whereas in research catches from 1925 to 1938, up to 60% of cod

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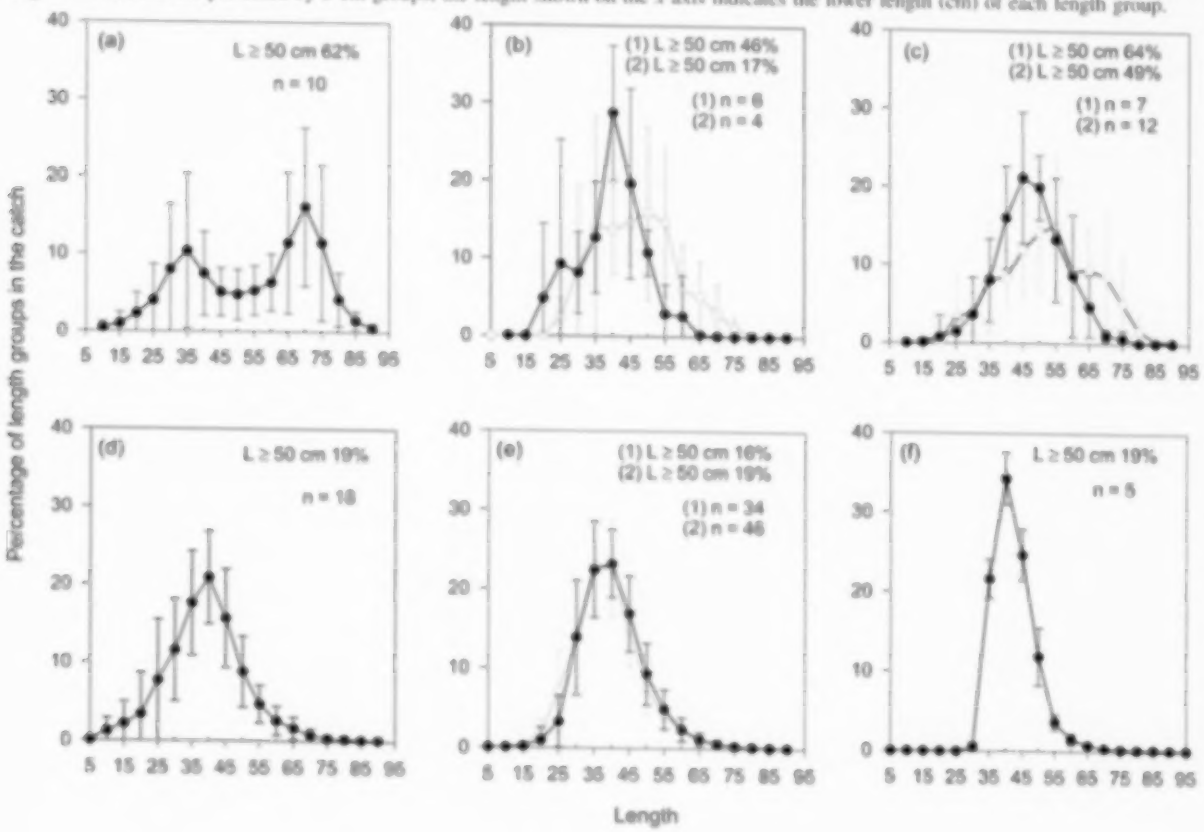
Fig. 3. Average length distributions of cod in (*a*) catches by RV *Poseidon* in 1925–1938, (*b*) catches by German cutters in 1938–1940 (1, open circles) and 1941–1944 (2, solid circles), (*c*) catches by German steamers in 1940–1941 (1, open circles) and 1942–1944 (2, solid circles), (*d*) catches by RV *Anton Dohrn* in 1962–1970, (*e*) commercial catches in 1947–1960 (1, open circles) and 1966–1975 (2, solid circles), and (*f*) Danish commercial catches in 2002–2006. Error bars represent variability in the data sets that were available by (*a*) year and area; (*b*) year and for some years by month; (*c*) year and in some years by area and (or) month; (*d*) year and area; (*e*) year, country, and subdivision; and (*f*) year. Each panel shows the number of available data sets (*n*) and the average percentage of fish ≥ 50 cm in length. The length distributions are presented by 5 cm groups; the length shown on the *x* axis indicates the lower length (cm) of each length group.



was ≥50 cm in length, similarly to the length structure of commercial catches by steamers in 1940–1941.

The condition of cod during the war was indicated to have been lower compared with the recent 15-year period. Fulton's condition factor in SD 25 in the first quarter of 1943 was estimated at 0.80, while the average in the years 1991–2007 in the equivalent area and season was estimated at 0.97.

Mortality estimates from catch curve analyses

Total mortality (*Z*) of cod in SDs 25–32 in the years 1940–1941 was estimated at 0.44 and increased to 0.81 in 1942–1944 (Table 3). The slopes of the catch curves for the years 1940–1941 and 1942–1944 were found significantly different ($t_{[2,8]} = 3.746$; $p < 0.01$). The corresponding SSB in 1940–1941 was estimated at around 300 thousand tonnes, and thereafter it declined to 113 thousand tonnes in 1944.

Length-based mortality estimates for the years 1953–1960 and 1966–1975 for SDs 26 and 28 were in line with the results from VPA and with the mortalities estimated from age-based catch curve analyses for the same time periods (Table 3). For SD 25, the average length-based total mortalities were estimated at 1.0 and 1.2 in 1953–1960 and 1966–1975, respectively. These values suggest about 0.2 units lower mortality in both periods than that obtained from the VPA (Table 3).

Analyses of CPUE

The average catch rate of RV *Poseidon* in SD 25 in 1925–1926 was 102 kg·h^{-1}. Similar catch rates were recorded in the years 1933–1935 and 1938 (91 and 126 kg·h^{-1}, respectively), while in 1929–1931 the average CPUE was three–four times lower (Fig. 4). The catch rates by RV *Poseidon* were at a similar level as the catches per hour by RV *Solea* in the early 1990s and by RV *Anton Dohrn* in the mid-1960s (Fig. 4), whereas the catchability of the RV *Poseidon* should be considered as the lowest among the three vessels (Table 2). The average SSBs in SDs 25–32

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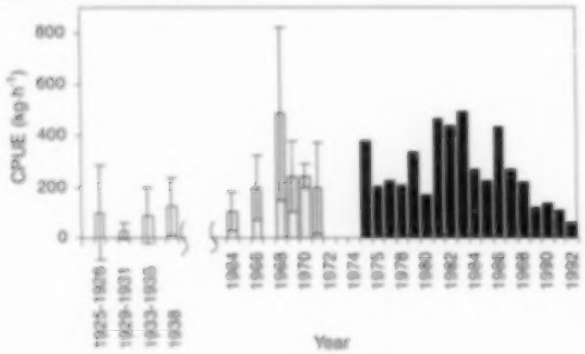
Table 3. Estimates of total mortality (Z) and confidence intervals based on length-converted catch curve analyses in comparison with total mortalities from age-based catch curve analyses and from virtual population analysis (VPA; Eero et al. 2007*a*) using extended survivors analysis (XSA) method.

Years	Area	XSA Z (ages 4–7)	Age-based catch curve Z (ages 4–7)	Length-converted catch curve Z (45–70 cm)
1940–1944	SDs 25–28	—	—	0.67 (0.62–0.73) ±0.20 (0.17–0.23)
1940–1941	SDs 25–28	—	—	0.44 (0.41–0.48) ±0.36 (0.31–0.40)
1942–1944	SDs 25–28	—	—	0.81 (0.75–0.89) ±0.21 (0.17–0.25)
1953–1960	SD 25	1.16	1.04±0.43	1.01 (0.94–1.10) ±0.05 (0.04–0.06)
1966–1975	SD 25	1.37	1.36±0.54	1.18 (1.11–1.29) ±0.02 (0.01–0.03)
1947–1956	SDs 26 and 28	1.05	1.05±0.19	1.12 (1.02–1.19) ±0.15 (0.13–0.16)
1966–1975	SDs 26 and 28	1.09	1.14±0.24	1.08 (0.98–1.15) ±0.07 (0.06–0.08)

Note: The range of the mortality estimates and confidence intervals obtained applying different von Bertalanffy growth parameter values are shown in parentheses. SD, ICES subdivision.

Fig. 4. Average catch per unit effort (CPUE, kg·h^{-1}) by RV *Poseidon* (Kändler 1944) in the period from 1925 to 1938, by RV *Anton Dohrn* (Tiews 1974; K. Tiews, Institute of Sea Fisheries, Palmaille 9, 22767 Hamburg, Germany, unpublished data) in the period from 1964 to 1971, and by RV *Solea* (Thurow and Weber 1992) in 1975–1992 in SD 25, represented by open, grey-shaded, and solid bars, respectively. Error bars represent variability in the data sets that were available by stations.



based on the catch rates of RV *Poseidon* were estimated at 155, 44, 138, and 191 thousand tonnes in the years 1925–1926, 1929–1931, 1933–1935, and in 1938, respectively.

Development of cod landings in the northern Baltic Sea and in the Gulf of Riga

Swedish cod landings in SDs 29–31 were between 2 and 20 t in the years 1925–1939 and rose to 350 t in the early 1940s (Fig. 5). The lowest landings in this period were recorded in the early 1930s, similar to the low level of landings in recent years (Table 4). The landings in the mid-1920s and late 1930s were comparable with the amounts of cod taken in these areas in the early 1970s when the average SSB in SDs 25–32 was between 200 and 225 thousand tonnes (Table 4). Estonian cod landings in SDs 29 and 32 increased from 50 t in 1934 to over 800 t in 1938. For comparison, in recent years the catches from this area have been negligible (Fig. 5). Similar average landings as in the years 1934–1935 and 1936–1938 were taken from this area in 1989–1991 and in 1973–1977, respectively. The corresponding average SSB in SDs 25–32 was between 200 and 300 thousand tonnes. The average Latvian landings in the Gulf of Riga in the years 1929–1931 were 7 t. Similar low landings were recorded in the years 1990–1992 when the SSB was around 150 thousand tonnes. The landings in the late 1930s were about 35 times higher, corresponding to the level of landings in the first half of the 1970s at an average SSB of 240 thousand tonnes (Table 4).

Egg abundances

The abundances of cod eggs in SD 25 in 1926, 1931, and 1938 were most similar to egg abundances observed in 1997, 1992, and 1976, respectively, when considering the time series from 1966 to 1999. The corresponding SSB values were 146, 92, and 355 thousand tonnes (ICES 2007), respectively (Table 5).

Comparison of the indications of SSB and corresponding fishing mortalities

The three estimates of SSB for the mid-1920s were between 150 and 200 thousand tonnes. The estimates for the late 1920s and early 1930s indicated a lower level of SSB, between 45 and 150 thousand tonnes. In the late 1930s, the SSB estimates were in the range of 190 to 355 thousand tonnes (Fig. 6). The lowest estimates in each time period were generally obtained from comparing catch rates of RVs *Poseidon* and *Solea*. The highest level of SSB in the late 1930s was indicated from egg abundance data, similar to the estimate from catch curve analyses for the early 1940s (Fig. 6).

Fishing mortalities were estimated on the basis of the ratio between total cod landings and SSB, except from the catch curve analyses, where mortalities were used to calculate biomass values. Fishing mortalities were below 0.2 from the mid-1920s until the mid-1930s, increased to 0.3 by the early 1940s, followed by a sharp increase to 0.6 during the war (Fig. 6).

Discussion

Historical development of the cod fishery and perception of the stock size

Technological developments in the Baltic Sea fisheries occurred much later compared with those in, e.g., the North Sea. Trawling was conducted by steamers in the North Sea already in the 19th century (e.g., Haines 1998), while in the Baltic Sea, motorization of boats only started in the begin-

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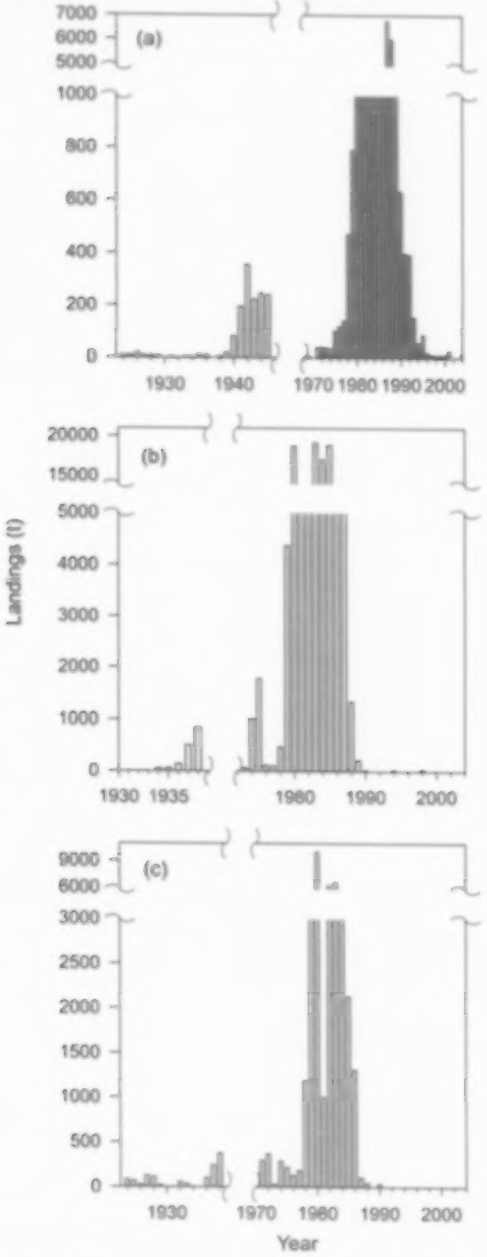
Fig. 5. Cod landings by (*a*) Sweden in SDs 29–31, (*b*) Estonia in SDs 29 and 32, and (*c*) Latvia in the Gulf of Riga.



ning of the 20th century, and trawls were introduced in the 1920s (Hessle 1947; Bager et al. 2007; Eero et al. 2007*b*). Trawls were initially small and adopted to catch flatfish that gave high yields in the 1920s and early 1930s (Molander 1955; Jensen 1959; Temming 1989). After severe reduction of flatfish catches in the mid-1930s, the trawls became larger and better adapted to catch cod (Hessle 1947; Sahlin 1959). Until then, cod was mainly caught by handlines and long lines (Hessle 1947; Jensen 1954).

With the Second World War, Germany lost its offshore fishing grounds in the Northeast Atlantic and had to confine the fishery to more coastal areas, i.e., southern part of the North Sea, Skagerrak, Kattegat, and the Baltic Sea (Meyer 1952*a*). It was seen as a crisis for the fishing industry, as before the war these areas were of very little importance for the fishery by steamers because of low profitability. In the Baltic Sea, a commercial fishery by steamers had so far not taken place, except for unsuccessful trials during the First World War. The Baltic Sea generally had a reputation as an overfished and low fish abundance area. This concept developed mainly in relation to the decline of flatfish stocks that were considered to be depleted because of the development of the trawl fishery (Meyer 1952*a*). In contrast with their expectations, the German steamer fleet found abundant resources of cod in the eastern Baltic (SDs 25–28) that formed a major part of total German landings during the war. The steamers' cod fishery was accompanied by scientific investigations from the beginning, first of all related to economical interests (Meyer 1952*a*, 1952*b*, 1952*c*). Consequently, although it may appear surprising, the first extensive materials related to the commercial fishery of eastern Baltic cod are from the investigations conducted during the war, and these provide important information for our study.

The cod fishery by steamers during the war changed the perception of the Baltic Sea in terms of its productivity and fish abundance. Although an increase in cod landings in the local fisheries was observed already in the late 1930s (e.g., Eero et al. 2007*b*), their level was still much lower compared with the landings by German steamers during the war. After the war, the cod fishery in the Baltic developed rapidly (e.g., Lablaika et al. 1991), and in the 1950s the exploitation rate was already at a high level (Eero et al. 2007*a*). Research on status of fish stocks in the Baltic developed in the eastern countries shortly after the war, but was mainly focusing on predicting future fishing possibilities, not on historical developments or their causes. The first attempt to estimate the stock size of the eastern Baltic cod in the early decades of the 20th century was made by Thurow (1999). Based on the results from his study and on the landings statistics, the cod stock is generally considered to have been at a very low level until it started to increase in the late 1930s. Consequently, the current understanding of the long-term impacts of major human-induced changes in the Baltic ecosystem (e.g., eradication of marine mammals, eutrophication) on fish populations is based on the perception of very low cod stock in the early 20th century (e.g., Österblom et al. 2007). However, the level of landings in the period when the fishery was undeveloped and when cod fishing effort was low (e.g., Kändler and Thurow 1959; Bagge et al. 1994) may not represent the level of the stock size (MacKenzie et al. 2002). Furthermore, the biomass estimates by Thurow (1999) before the 1940s are based on few data and rely on rough assumptions (e.g., the mortality estimates of the population are largely based on data from young fish surveys, which did not catch larger fish; Thurow 1997*b*) that inevitably lead to uncertain estimates. Therefore, the current perception of the development of cod stock and fish productivity of the Baltic ecosystem before the 1950s needs to be evaluated by alternative approaches, such as those used in this investigation.

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Table 4. Average cod landings by Sweden in ICES subdivisions (SDs) 29–31, by Estonia in SDs 29 and 32, and by Latvia in the Gulf of Riga in the mid-1920s, in the late 1920s to early 1930s, and in the late 1930s to early 1940s compared with the corresponding levels of average landings and spawning stock biomasses (SSB) in the period after the 1970s.

Area	Years	Landings (t)	Years	Landings (t)	SSB (10^3 t)
SDs 29–31	1925–1927	13	1969–1971	15	205
	1930–1932	2	2002–2004	4	81
	1938–1940	36	1972–1974	37	225
SDs 29 and 32	1934–1935	59	1989–1991	82	202
	1936–1938	500	1973–1977	667	299
Gulf of Riga	1929–1931	7	1990–1992	11	153
	1936–1938	240	1971–1975	239	240

Table 5. Cod egg abundances (individuals·m^{-3}) and corresponding salinities (psu, 80 m depth) in Bornholm Basin in the years 1926, 1931, and 1938 and spawning stock biomasses (SSB, 10^3 t) in the period from 1966 to 1999, in the years when the egg abundances were similar to the values observed in 1926, 1931, and 1938.

	Year		
	1926	1931	1938
Egg abundance, salinity	39, ~16	11, ~15	89, 16.8
Egg abundance, salinity in comparative year	45, 15.8 (1997)	9, 14.9 (1992)	96, 16 (1976)
SSB	146	92	355

Uncertainties in the biomass estimates

A well-known general problem for estimating fish stock biomass based on commercial landings is the accuracy of reported statistics. In this study, total cod landings were utilized in one of the four methods, i.e., catch curve analyses covering the years 1940–1944. The cod fishery in the eastern Baltic Sea in these years was mainly conducted by German steamers, and their landings were closely monitored (Meyer 1952*a*). Therefore, potentially under-reported landings during these years are not expected to have a major impact on the results of this study.

One of the shortcomings with our catch curve analysis is the assumption of relatively constant year-class strength. The year-class strength of the eastern Baltic cod is partly related to hydrographic conditions (e.g., Jarre-Teichmann et al. 2000; Köster et al. 2001) that are determined by inflows of North Sea water into the Baltic Sea (Schinke and Matthäus 1998). The average inflow data (Matthäus 2006) for the years corresponding to hatching of the year classes appearing as 4- to 7-year olds in 1940–1941 and in 1942–1944 indicate that possible differences in year-class strength due to variable hydrographic conditions may have led to overestimation of mortality. In both periods, the oldest length-converted age group included in the catch curve estimates has, on average, been exposed to weaker inflow conditions at hatching compared with younger ages. These weaker year classes could have contributed to the steepness of the catch curve and consequently resulted in overestimation of mortality. In addition to relatively high uncertainty in the total mortality estimate for 1940–1941, the fishing mortality and biomass for this period were also sensitive to the assumption of natural mortality, as the level of total mortality was estimated to be relatively low. The natural mortality could have been higher taking into account predation mortality by marine mammals that were more abundant in this period compared with recent decades (Harding and Härkönen 1999). The length-based catch curve estimates also rely on the assumption that the growth of the fish was similar in the 1940s compared with the 1960s–1970s. Lower condition factor of cod in 1943 compared with recent decades indicates that the growth rates were likely not higher in the earlier period. If the growth rate was lower, e.g., because of lower food availability, the biomasses based on catch curve analysis are underestimated, similar to the effect of variable year-class strength and underestimated natural mortality.

Our biomass estimates based on comparison of catch rates from research surveys assume similar catchability of RVs *Poseidon* and *Solea*. This is a rough assumption, as catchabilities of vessels are known to differ. However, the catchability of *Poseidon* is not expected to have been higher compared with *Solea*. Although the trawl used by *Solea* was smaller in terms of groundrope length, this was likely compensated by modern technology and double horse power of the engine. The information on exact trawling speed of *Poseidon* is unavailable, but there are indications that it could have been low. During 1–2.5 h, it covered 2.5–5 nautical miles (1 n.m. = 1.853 km) in earlier expeditions (Strodtmann 1905), suggesting an average trawling speed of about 2 kn (1 kn = 1.853 km·h^{-1}); *Solea* trawled at 3–3.5 kn (ICES 1988). Therefore, at a similar stock size, the catch rates of *Poseidon* could hardly be higher than those of *Solea*, and our SSBs from catch rate analyses are likely underestimated rather than overestimated.

The landings in the northern Baltic Sea and in the Gulf of Riga in the 1920s–1930s were at a similar level to the landings in these areas in the 1970s and 1990s, while the total Baltic cod landings in the earlier period were about 10 times lower. This could be due to a different cod distribution pattern in the Baltic in the early decades, or it may indicate that fishermen were able to take similar landings in these rela-

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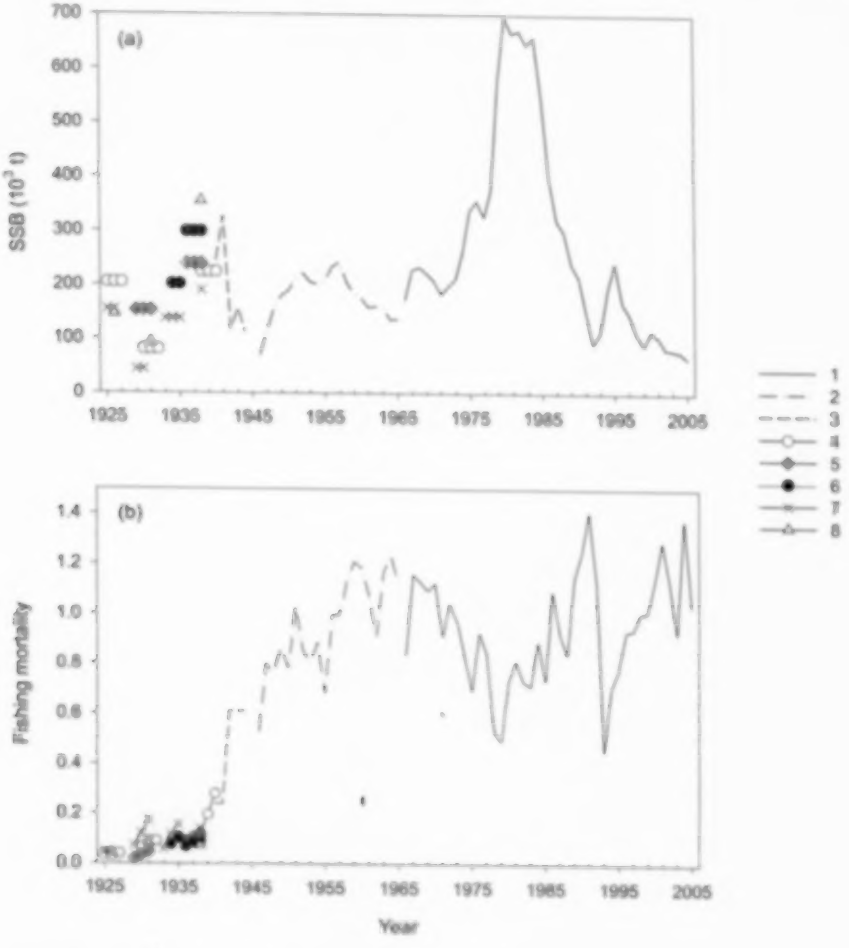
Fig. 6. Estimates of cod spawning stock biomass (SSB) (*a*) and fishing mortality (*b*) for the years 1925–2005 by different methods: 1, International Council for the Exploration of the Sea (ICES) assessment (ICES 2007); 2, virtual population analysis (VPA; Eero et al. 2007*a*); 3, based on mortality estimates from length-based catch curve analysis; 4, based on landings in ICES subdivisions (SDs) 29–31; 5, based on landings in the Gulf of Riga; 6, based on landings in SDs 29 and 32; 7, based on catch-per-unit-effort (CPUE) data; 8, based on egg abundances.



tively coastal areas, while the offshore areas were proportionally much less exploited. We have taken into account possible differences in stock distribution caused by variations in salinity (Lablaika and Lishev 1961; Tomkiewicz et al. 1998) by comparing only time periods with similar salinity conditions. Our analyses assume similar fishing effort in the two time periods, though the effort in the earlier period could be expected to have been lower. However, the differences in effort in these areas are likely not as large as those for the total Baltic Sea, as the higher effort in the cod fishery in the later period has mainly been directed to offshore areas, not to the nearshore areas of the northern Baltic or the Gulf of Riga. It should be noted that in some cases, especially concerning the SDs 29 and 32, the compared landings in the 1930s and in the period after the 1970s were not entirely similar. The lower landings in the 1930s indicate that the respective SSB value in the 1930s could have been overestimated.

Among the data used in comparisons between the 1920s–1930s and the more recent decades, the egg abundances should be considered as the most comparable in terms of technological developments. Catchability differences between the standard gear used by the former USSR and later by Latvia, i.e., the IKS-80, and the standard gear used by Denmark and Germany since the early 1970s, i.e., a bongo net, are negligible (CORE 1998). As earlier German sampling was conducted with a gear similar to the IKS-80, it can be assumed that catchability differences are marginal between all three gears used for sampling ichthyoplankton in the 20th century. The egg abundances for the period from 1966 refer to the main 3-month spawning season, whereas this may not be the case with the earlier data. A mismatch of spawning season and sampling time would result in an underestimation of the spawner biomass in earlier years. As the conducted comparison is based on egg abundances, i.e., integrating over all egg developmental stages, a

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pronounced difference in egg mortality would affect the validity of the approach as well. However, as only periods with similar hydrography, i.e., salinity, were compared, large differences in salinity-related egg mortality are not expected. Another factor potentially decoupling egg abundance and spawning stock size is fecundity that is related to prey availability (Kraus et al. 2002). Based on the estimation for the early 1940s, the condition of cod in the late 1930s was likely lower than that in the 1990s. Therefore, it appears to be unlikely that fecundity was higher and consequently the spawner biomasses at least in the mid-1920s and early 1930s that are based on the egg data from the 1990s are underestimated rather than overestimated. A factor that may result in an overestimation of spawner biomass, however, is the fact that in a stock characterized by the presence of old fish, the proportion of female cod will be higher (Tomkiewicz et al. 1997), and consequently the egg production per unit of spawner biomass will as well be higher.

The level of stock size and fishing intensity

The SSB of cod in the mid-1920s and in the period from the late 1930s to the beginning of the 1940s was estimated about two–five times higher compared with the current stock size. The estimates for the late 1920s to early 1930s were in a similar range as the SSB in recent years. The highest SSB during 1925–1944, up to 350 thousand tonnes, was estimated for the late 1930s to early 1940s, similar to the level in the mid-1970s and in the mid-1980s. The spawning stock could have been close to this level also in the mid-1950s, as the stock size in SD 25 in this period from the VPA was considered underestimated (Eero et al. 2007*a*). This is further confirmed by our length-based catch curve analyses indicating lower fishing mortality in SD 25 in the 1950s–1970s than that obtained from age-based VPA in a previous study. This can be explained by the fact that the available length data represented a larger part of the catches in SD 25 compared with the age-specific data utilized by Eero et al. (2007*a*).

The relatively high level of stock size in the 1930s to early 1940s as estimated here is supported by additional indicators. Abundance of cod larvae in the 1930s was similar to the 1960s to early 1970s and much higher compared with the 1990s (MacKenzie et al. 1996). Relatively high cod biomass in the early 1940s is also supported by cod stomach investigations. Cod cannibalism, which is generally related to high stock abundance (Neuenfeldt and Köster 2000), was observed during the Second World War in all central Baltic areas (Meyer 1952*c*). Tiews (1974) compared the catch rates of RV *Anton Dohrn* in the 1960s with those of German steamers during the Second World War and found that the catch rates in 1962–1970 in Bornholm Basin and also in 1962–1968 in Gdansk and Gotland areas were generally lower or at the same level as during the war. The fishing gear of the vessels is reported to have been similar (Meyer-Waarden and Tiews 1967); however, it should be noted that the capacity (BRT) of the steamers fishing in the Baltic Sea during the war was about two or three times lower compared with RV *Anton Dohrn* (Meyer 1952*a*). The total fish biomass in the Baltic during the war is also indicated to have been higher than that in neighboring areas. Daily catch rates (all species) during the war were about twice as high in the Baltic as in the southern North Sea, Skagerrak, and Kattegat (Meyer 1952*a*). Also, average catch rates of all fish species in the total North Sea and in Skagerrak before the war were lower than those in the Baltic during the war, not taking into account the North Sea herring fishery (Meyer 1952*a*).

Our results concerning the reduction in cod stock at the end of the war were supported by comparison of the catch rates of steamers during the war and the catch rates of cutters in the following years. The catch rates after the war were about two times lower, while the fishing power of these fleets was considered comparable (Meyer 1952*a*). The estimated level of stock size at the end of the war is also in line with results from an analytical assessment (Eero et al. 2007*a*) for the second half of the 1940s. The reduction in cod stock size in the first half of the 1940s corresponds to a sharp increase in fishing mortality in preceding years, and the catch rates declined from 1940 to 1944 (Meyer 1952*a*). The impact of fishing on the cod population during the war is also indicated by the change in size composition of catches. In addition to sampled length distributions, a reduction in the proportion of larger cod in the landings during the 1940s is evident also in the market categories of total landings. The proportion of cod in the category of large fish (above 40 cm) nearly halved during the war in all fishing areas of the central Baltic Sea (Meyer 1952*b*). In addition to the fishery by German steamers on relatively large cod, Sweden carried out an intensive fishery with small-meshed trawls catching large quantities of small cod (Alander 1946). According to observations by Alander (1946), the mean weight of cod in Swedish catches during the war years was only 100–200 g. This indicates that the intensive fishery on young cod contributed to the fishing pressure on the stock in addition to the fishing mortality on larger cod that was estimated to be at a moderate level at the end of the war. Heavy fishery was therefore likely the major factor contributing to the reduction in stock size in the first half of the 1940s. The Baltic cod, which likely for the first time in history was intensively exploited during the Second World War, thereby provides a contrasting example to many other fish stocks in the Northeast Atlantic, which were almost not exploited during the war (Pope and Macer 1996; Hylen 2002), and some consequently increased in abundance (e.g., Rijnsdorp and Van Leeuwen 1992).

The fishing mortality on the eastern Baltic cod in the 1920s–1930s was estimated below 0.2, which is much lower than the mortality rate on cod, e.g., in the North Sea (0.5) in the same time period (Pope and Macer 1996). Lower fishing pressure in the Baltic Sea at this time is in line with the much less developed fishing technology in the Baltic Sea compared with the North Sea. Despite the low fishing pressure, the level of stock size in the Baltic before the war was estimated to be lower than that observed in the late 1970s–1980s. The Baltic thereby differs from many other areas where fish abundances before the beginning of intensive fishery were much higher than observed since then (e.g., Jackson et al. 2001; Myers and Worm 2003). Relatively low fish biomass in the Baltic before the onset of an intensive fishery is likely related to lower nutrient concentrations in the Baltic ecosystem that gradually started to increase after the Second World War (Elmgren 1989). The net

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biomass productivity in the 1920s–1930s was estimated to be a factor of 2.5 lower compared with the recent decade (Schneider and Kuss 2004). The food limitation for cod in the early 1940s is also indicated by the relatively low condition factor. Additionally, the abundances of potential cod predators, i.e., seals, were higher in the 1920s–1930s compared with the later period (Harding and Härkönen 1999).

In summary, our spawning stock estimates are based on different proxies that vary in precision. Several of our analyses were considered to underestimate the SSB in the 1920s–1930s. Therefore, the true stock might have been larger than shown here; however, based on present knowledge, there are no indications that it could have reached the level close to the highest recorded biomass in the early 1980s. The level of landings by a relatively intensive fishery during the war was still about five times lower than that in the late 1970s to early 1980s, while the impact of the fishery on the stock during the war at this level of landings was obvious. The multiple proxies are consistent in terms of the level of stock size in each time period, which increases the confidence in results. However, the exact biomass values should be treated with suitable caution.

Our study demonstrates that cod biomass in the Baltic Sea in the 1920s–1940s was higher and showed different trajectory than previously believed. The fishing mortality was low until the Second World War, when it increased sharply to moderate levels. The biomasses in the earlier years therefore provide a new baseline for the level and variability of stock size at low fishing pressure and improve the basis for estimating the relative roles of multiple human impacts and climate on the long-term development of the cod population.

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A Bayesian approach to identifying mixtures from otolith chemistry data

S.B. Munch and L.M. Clarke

Abstract: Studies investigating population structure and mixed-stock composition of fish populations frequently use otolith chemistry as a natural tool for discerning stocks. Current methods for estimating mixed-stock composition, however, assume complete accuracy in the training data, which is often not the case. Here we present a method for estimating mixed-stock composition using multivariate continuous data that accounts for uncertainty in the training data. Application of the method to previously reported data for natal homing in weakfish (*Cynoscion regalis*) and simulations based on these data revealed that for sample sizes greater than about 30, the present method provides results that are quite similar to those of previous methods. An advantage of this Bayesian approach over other methods, however, is the ease with which functionals of the model, such as migration distance and direction, can be calculated. It also provides simple means of visualizing spatial structure in the classification probabilities and migration patterns.

Résumé : Les recherches qui s'intéressent à la structure de population et à la composition des stocks mixtes chez les populations de poissons utilisent souvent la chimie des otolithes comme outil naturel pour séparer les stocks. Cependant, les méthodes courantes pour estimer la composition des stocks assument que les données d'entraînement sont parfaitement exactes, ce qui n'est souvent pas le cas. Nous présentons ici une méthode pour estimer la composition de stocks mixtes qui utilise des données multidimensionnelles continues qui tiennent compte de l'incertitude dans les données d'entraînement. L'utilisation de la méthode avec des données publiées antérieurement sur le retour au lieu de naissance chez l'acoupa royal (*Cynoscion regalis*) et des simulations basées sur ces données montrent que, pour des échantillons supérieurs à environ 30, notre méthode fournit des résultats qui sont très semblables à ceux des méthodes antérieures. Un avantage de notre approche bayésienne par rapport aux autres méthodes est la facilité avec laquelle on peut calculer les fonctionnelles du modèle, telles que la distance et la direction de la migration. Elle procure aussi un moyen simple de visualiser la structure spatiale dans les probabilités de la classification et les patrons de migration.

[Traduit par la Rédaction]

Introduction

The chemical composition of otoliths serves as a natural tag for the investigation of population structure and connectivity in fish populations. As otolith material is deposited, trace elements and stable isotopes are incorporated into the calcium carbonate structure, creating a chemical fingerprint (Thresher 1999). Because different natal habitats often create unique otolith fingerprints, otoliths can be used to determine the natal origin of adult fish (reviewed in Campana and Thorrold 2001). Consequently, otoliths have been used to determine patterns of natal homing (Thorrold et al. 2001), estimate recruitment sources (Gillanders and Kingsford 1996; Hamer et al. 2005; Wright et al. 2006), examine connectivity between juvenile and adult habitats (Gillanders 2002), and investigate the degree of mixing among populations (Campana et al. 1999, 2007). Each of these objectives requires the use of mixed-stock analysis to identify the proportions of various stocks comprising the sample catch.

Mixed-stock analysis attempts to estimate the fraction of individuals coming from different sources for a given mixed population. The estimation problem is typically broken into three parts. First, a set of individuals of known origin is analyzed to develop a classification rule. Second, a classification step is performed to determine the sources of origin for each individual in the mixed sample. Conditional on this classification, a third step is performed to obtain the maximum likelihood estimates of the mixing proportions (Millar 1987, 1990).

Most mixed-stock applications in fisheries are typically based on morphometric, meristic, or genetic data (Fournier et al. 1984). Differences in vertebral number (Swain et al. 2001), scale patterns (Bernard and Myers 1996; Reddin and Friedland 1999), otolith morphology (DeVries et al. 2002), and a variety of other morphometric and meristic characteristics (Waldman et al. 1997) have been used to distinguish various fish stocks from one another and determine mixed-stock composition. Although genetic data have been the most common means of assessing population structure, otolith chemistry studies are becoming more frequent. This increase is most likely due to the ability of otoliths to detect

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structuring not always evident in genetic data (Campana and Thorrold 2001; Palumbi 2004).

Otolith chemical signatures are inherently multivariate. The elemental composition often results from the substitution of various trace elements for calcium in the otolith crystal lattice (Campana 1999). Both environmental and physiological processes can influence elemental otolith concentrations, and this can sometimes lead to strong correlations among trace elements. Discriminant function analysis and maximum likelihood based on multivariate normality are the most commonly applied methods for classification and mixed-stock analysis using otolith microchemistry (White and Ruttenberg 2007), although machine learning methods such as neural networks and support vector machines have also been used (Thorrold et al. 1998; Castro 2007). Unfortunately, otolith chemistry studies are still exceedingly expensive, so sample sizes are typically quite limited. As a consequence, the training data used to estimate the classification rule are also typically small and consequently subject to some uncertainty.

As Pella and Masuda (2001) point out, the standard methods for mixed-stock analysis based on genetic data typically assume that the training data and constructed classification rule are known without error. This caveat applies to the discriminant function and maximum likelihood analyses typically used in otolith studies as well. To circumvent this problem for discrete genetic data, Pella and Masuda (2001) developed a Bayesian method that also allows for simultaneous classification and estimation of mixing proportions. Fully Bayesian approaches to classification based on genetic data have been shown to outperform other methods (Manel et al. 2002). Here we develop an analogous Bayesian mixed-stock approach suitable for continuous multivariate data.

There are now many papers on the relative merits of frequentist and Bayesian analyses in ecological research (Dennis 1996; Ellison 2004; Stephens et al. 2007). We have little desire to add to this list but summarize the main differences here. In frequentist statistics, parameters are fixed and probability is interpreted in the classical sense. In Bayesian statistics, parameters are random and the subjectivist interpretation of probability is used. Given that parameters are random under a Bayesian view, what is known about them is summarized by probability models that, given new data, are updated using Bayes' rule. In data-limited situations, this inclusion of prior information may strongly influence statistical analysis. However, for a wide range of problems, if the priors are vague and the data are informative, Bayesian and frequentist estimates converge on the same numerical values. Thus, in most cases of scientific relevance, these differences are mainly philosophical. From our viewpoint, the chief advantages of Bayesian methods over analogous frequentist approaches are the ease with which multiple sources of information may be brought to bear on a single question, the ease with which different types and sources of uncertainty may be accounted for, and the simplicity with which uncertainty may be propagated when making predictions.

To illustrate the utility of the method and compare it with prior approaches, we apply the method to previously analyzed data on weakfish (*Cynoscion regalis*; Thorrold et al. 2001).

Materials and methods

Our goal is to develop a method for identifying population mixtures based on otolith chemistry data that accounts for uncertainty in the training data. In keeping with the otolith chemistry literature, we assume that the otolith signatures have, on some scale, a multivariate normal distribution that differs hopefully in mean and possibly in covariance at each location (this assumption is equivalent to the commonly used quadratic discriminant function). Specifically, for a sample known to originate from site s, the likelihood of observing an otolith signature $\boldsymbol{x}$ is denoted by $p(\boldsymbol{x}|\boldsymbol{\mu}_s, \boldsymbol{\Sigma}_s)$ and is given by the multivariate normal density $N_q(\boldsymbol{\mu}_s, \boldsymbol{\Sigma}_s)$. Here q is the number of elements in $\boldsymbol{x}$, $\boldsymbol{\mu}_s$ is the population average or expected signature for fish from site s, and $\boldsymbol{\Sigma}_s$ is the covariance matrix for signatures drawn from this site.

As in all classification problems, the data are divided into two subsets. The first, $\mathbf{X}$, consists of samples of known origin collected from S different sites and is referred to as the training data. The second, $\mathbf{Y} = [\boldsymbol{y}_1, \ldots, \boldsymbol{y}_n]$, is referred to as the sample data that contains a mixture of individuals from several sites. The analytical goal is to estimate the composition of the sample data, i.e., to estimate the proportions of fish from each site of origin found in the sample data, denoted by $\boldsymbol{\Theta} = [\theta_1, \ldots, \theta_S]$. We do so in several steps, which are outlined here and described in more detail below. First, we establish uninformative priors for the site-specific parameters $\boldsymbol{\mu}_s$ and $\boldsymbol{\Sigma}_s$, as well as for the mixture proportions $\boldsymbol{\Theta}$. Previous classification methods for otoliths set $\boldsymbol{\mu}_s$ and $\boldsymbol{\Sigma}_s$ equal to the sampled mean and covariance. Although this is certainly reasonable for large sample sizes, for the relatively small samples typically available in otolith studies, this approach neglects sampling uncertainty in these parameters. Second, we update the distributions for $\boldsymbol{\mu}_s$ and $\boldsymbol{\Sigma}_s$ based on the training data to obtain $p(\boldsymbol{\mu}_s, \boldsymbol{\Sigma}_s|\mathbf{X}_s)$ and determine the posterior predictive distribution $p(\boldsymbol{y}_i|\mathbf{X}_s)$ by integrating out the unknown mean and covariance. This distribution provides us with an updated likelihood for otolith signatures from each site that has fatter tails than the normal distribution commonly used in otolith studies. Finally, with $p(\boldsymbol{y}_i|\mathbf{X}_s)$ in hand, we then use the sample data to simultaneously estimate the site of origin σ_i for each individual and the mixing proportions $\boldsymbol{\Theta}$ for each site.

We begin by establishing vague priors for $\boldsymbol{\mu}_s$, $\boldsymbol{\Sigma}_s$, and $\boldsymbol{\Theta}$. There are many ways in which uninformative priors may be constructed. Uniform priors, Jeffreys priors (Jeffreys 1961), reference priors (Bernardo 1979), and maximum entropy priors are typical choices that attempt to impart the least prior information. Jeffreys priors have the desirable property that the resulting posterior is invariant to scale transformations, although these can be difficult to work with in high dimensional problems (Dawid et al. 1973). For sake of analytical simplicity, we use a flat prior for $\boldsymbol{\mu}_s$ and a vague Wishart prior for $\boldsymbol{\Sigma}_s$. For the $\boldsymbol{\Theta}$ prior, we use a nearly uniform Dirichlet distribution. As noted below, this specification is effectively identical to choosing independent Jeffreys priors for $\boldsymbol{\mu}_s$, $\boldsymbol{\Sigma}_s$, and $\boldsymbol{\Theta}$. More details on these distributions can be found in Gelman et al. (1995). Although other priors are certainly possible, these conjugate priors were chosen because they allow us to do nearly all of the posterior updating analytically and require little modification to include

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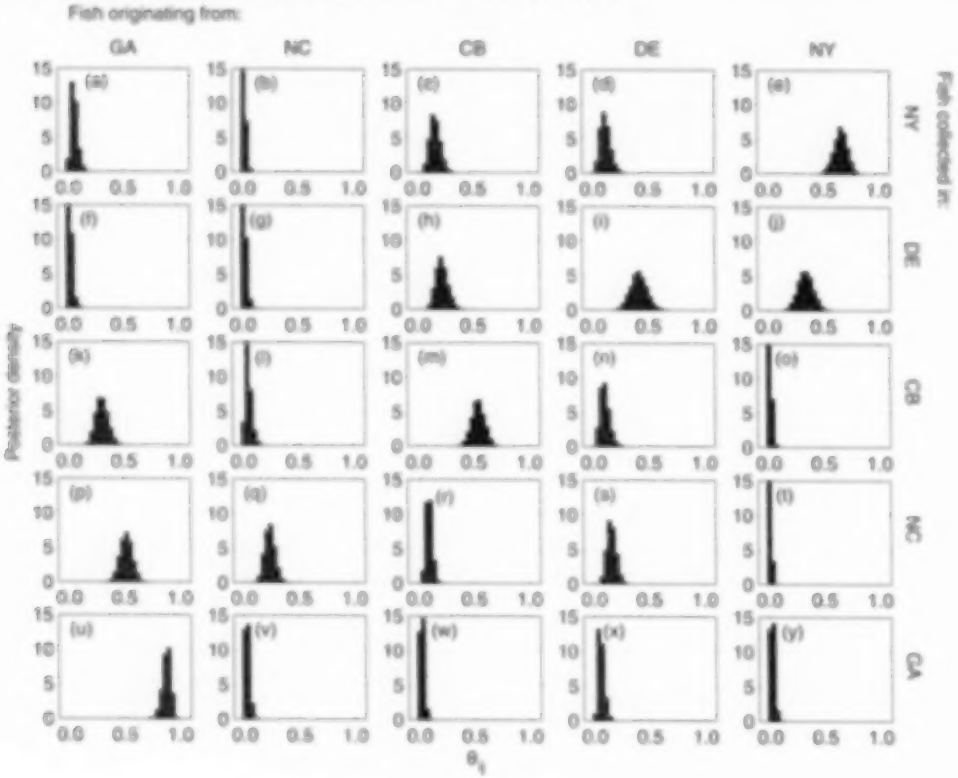
Fig. 1. Posterior for $\boldsymbol{\Theta}$. Each row corresponds to a collection site and each column corresponds to a putative site of origin. For each row, histograms give the marginal posterior probability for the mixing fractions for each site: (*a*–*e*) New York, (*f*–*j*) Delaware, (*k*–*o*) Chesapeake Bay, (*p*–*t*) North Carolina, and (*u*–*y*) Georgia. Note that the panels on the diagonal (lower left to upper right) give the probability that fish sampled in a region also originated there (i.e., were juveniles in the same location). The off-diagonal panels for each row indicate the posteriors for the proportion of individuals that originated in another location, i.e., migrants.



additional prior information. Our three-component prior is given by

$$(1) \qquad p(\boldsymbol{\mu}_s) \propto 1$$

$$(2) \qquad p(\boldsymbol{\Sigma}_s) = \left[2^{\nu q/2}\Gamma_q\left(\frac{\nu-1}{2}\right)\right]^{-1}|\mathbf{W}|^{\nu/2}|\boldsymbol{\Sigma}_s|^{-(\nu+q+1)/2} \times \exp\left[-\frac{1}{2}\mathrm{tr}(\mathbf{W}\boldsymbol{\Sigma}_s^{-1})\right]$$

$$(3) \qquad p(\boldsymbol{\Theta}) = \Gamma\left(\sum\alpha_s\right)\left[\prod_{s=1}^{S}\Gamma(\alpha_s)\right]^{-1}\prod_{s=1}^{S}\theta_s^{\alpha_s-1}$$

where ν is the prior degrees of freedom and $\mathbf{W}\nu^{-1}$ is a prior guess at the covariance matrix. $\boldsymbol{\alpha} = \{\alpha_1, \ldots, \alpha_S\}$ is proportional to the prior expectation for the elements of $\boldsymbol{\Theta}$. In the absence of any real information about these parameters, we allow $\nu \to 0$ and set $\mathbf{W} = r\mathbf{I}_q$ with r given by the maximal range in the otolith signatures. $\mathbf{I}_q$ is the $q \times q$ identity matrix. In this limit, the prior for $\boldsymbol{\Sigma}_s$ is proportional to $|\boldsymbol{\Sigma}_s|^{-(q+1)/2}$, which is the Jeffreys prior for the covariance matrix.

We set all of the α_ss = 1 such that $p(\boldsymbol{\Theta})$ is uniform. Jeffreys prior for $\boldsymbol{\alpha}$ is also Dirichlet, with $\alpha_s = 1/2$, which has the same prior mean as our model (i.e., 1/number of sites) but greater variance attained by concentrating probability near $\theta = 0$ and $\theta = 1$. When additional prior information is available, it may be worthwhile to contemplate distance-based priors for $\boldsymbol{\Theta}$, as well as spatially structured priors for $\boldsymbol{\mu}_s$ and $\boldsymbol{\Sigma}_s$. For instance, we might assert that the components of the mean vector for each sampling site were not independent but instead came from a multivariate distribution in which correlation between sites decays with distance (for a recent fisheries application, see, e.g., Su et al. 2004).

Before going any further, we must clarify the notation slightly. Let N_s indicate the number of individuals in the training data from site s and $\mathbf{X}_s = \{\boldsymbol{x}_{s1}, \ldots, \boldsymbol{x}_{sN_s}\}$ denote the training data from this site. As indicated earlier, $\mathbf{X}$ denotes the complete set of training data, which includes individual signatures from all sites, i.e., in the notation just introduced, $\mathbf{X} = \{\mathbf{X}_1, \ldots, \mathbf{X}_S\}$. Also, we will use $\bar{\boldsymbol{x}}_s$ and $\bar{\mathbf{C}}_s$ to indicate the sampled mean and covariance for the training data from

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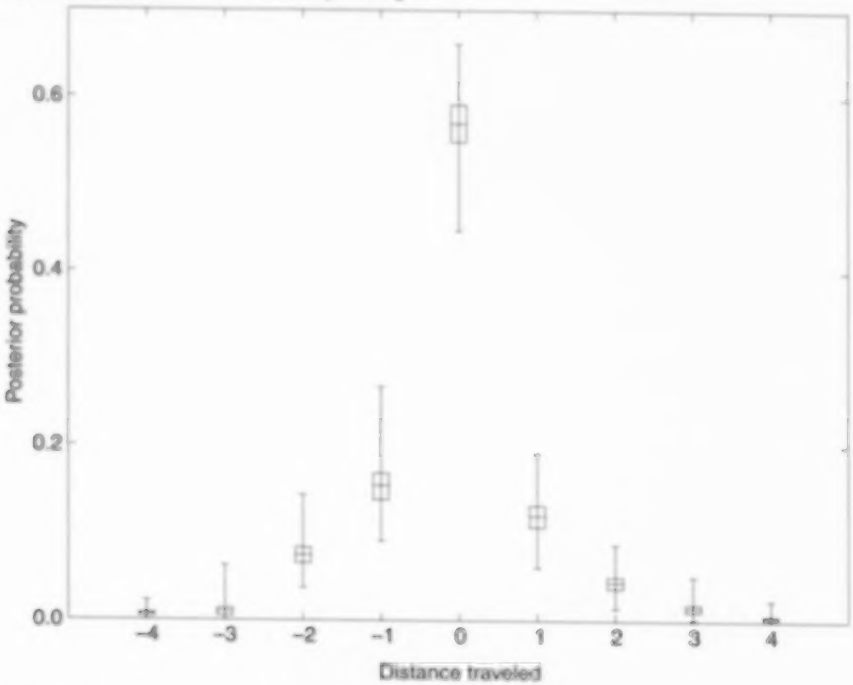
Fig. 2. Average migration kernel. The vertical axis gives the probability of moving a given distance independent of starting location. The horizontal axis is distance in numbers of estuaries, with positive values indicating northward movement. Box-and-whisker plots summarize the posterior probability of an individual moving a given number of estuaries averaged over starting locations. The center line for each box gives the location of the posterior mean, the top and bottom box edges give the upper and lower quartiles of the distribution, and the whiskers give the entire support for the posterior (i.e., the sampled range).



site s. Using the training data, our next step is to update the priors for $\boldsymbol{\mu}_s$ and $\boldsymbol{\Sigma}_s$ and integrate to obtain the likelihood for new observations conditional on the training data. Some details on these calculations are provided in Appendix A. Using σ_i to indicate the ith individual's site of origin, the end result is

$$(4)\quad p(\mathbf{y}_i|\sigma_i = s, \mathbf{X}_s) = \frac{\left(\frac{N_s}{\pi(N_s+1)}\right)^{p/2}\Gamma_q\left(\frac{N_s+\nu}{2}\right)|\boldsymbol{\Sigma}_s + \mathbf{W}|^{(N_s+\nu-1)/2}}{\Gamma_q\left(\frac{N_s+\nu-1}{2}\right)|\boldsymbol{\Sigma}_s + \mathbf{W} + \frac{N_s}{N_s+1}(\mathbf{y}_i - \bar{\mathbf{x}}_s)(\mathbf{y}_i - \bar{\mathbf{x}}_s)^T|^{(N_s+\nu)/2}}$$

which will serve now as the likelihood for the sample data given the training data and a putative site of origin s. Note that in traditional applications, a normal distribution based on the sampled mean and covariance would be used in place of eq. 4, which may have considerably heavier tails than the normal for small training data sets.

Our goal, finally, is to obtain the posterior distributions for the individual σ_is and the site-specific $\boldsymbol{\Theta}$s. Because the site of origin for any individual is unknown, it is assigned a prior probability distribution given by the mixing proportions $\boldsymbol{\Theta}$. That is, $\Pr(\sigma_i = s) = \theta_s$. This equation, along with eqs. 3 and 4, comprise the hierarchical model for the mixture problem. Although it is possible to obtain conditional distributions for the σ_is or $\boldsymbol{\Theta}$ (see, e.g., Geisser 1964; Millar 1987), we have found no way to obtain posterior marginals for both. However, an efficient Monte Carlo algorithm using Gibbs sampling can be obtained easily. (Gibbs sampling is a special case of the Metropolis–Hastings algorithm (Gelman et al. 1995) in which the full conditional distribution for each parameter, or block of parameters, is used as the proposal distribution, leading to an acceptance probability of 1. For more details on Gibbs sampling, see Gelman et al. (1995).) In the present case, having calculated and stored the likelihood that each individual came from each of the possible sites, i.e., $p(\mathbf{y}_i|\sigma_i = s, \mathbf{X})$ for $s = 1, \ldots, S$, we simply alternate between sampling the full conditional for σ_i, $p(\sigma_i|\boldsymbol{\Theta}, \mathbf{y}_i, \mathbf{X})$, and the full conditional for $\boldsymbol{\Theta}$, $p(\boldsymbol{\Theta}|\sigma_i, \mathbf{y}_i, \mathbf{X})$. These full conditionals are given by

$$(5)\quad p(\sigma_i = s|\boldsymbol{\Theta}, \mathbf{y}_i, \mathbf{X}) = \frac{\theta_s p(\mathbf{y}_i|\sigma_i = s, \mathbf{X})}{\sum_{j=1}^{S}\theta_j p(\mathbf{y}_i|\sigma_i = j, \mathbf{X})}$$

$$(6)\quad p(\boldsymbol{\Theta}|\boldsymbol{\sigma}, \mathbf{Y}, \mathbf{X}) = \Gamma\left(\sum \alpha_s + c_s\right)\left[\prod_{s=1}^{S}\Gamma(\alpha_s + c_s)\right]^{-1}\prod_{s=1}^{S}\theta_s^{\alpha_s + c_s - 1}$$

where $\boldsymbol{\sigma} = \{\sigma_1, \ldots, \sigma_n\}$ is the collection of individual sites and c_s is the number of these for which $\sigma_i = s$, i.e., $c_s = \sum_{i=1}^{n} 1_{|\sigma_i = s|}$.

One of the main advantages of moving to a Bayesian framework is the ease with which posterior distributions for

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functionals of the model parameters may be calculated. For instance, we might ask whether it is more likely for migrating weakfish to have come from the north or the south, or how far individuals are likely to have migrated. The probability that a migrant individual came from the north may be calculated as

$$(7)\quad P(\text{from north}|\mathbf{Y},\mathbf{X}) = \int P(\text{from north}|\boldsymbol{\Theta})p(\boldsymbol{\Theta}|\mathbf{Y},\mathbf{X})\mathrm{d}\boldsymbol{\Theta}$$

where $P(\text{from north}|\boldsymbol{\Theta}) = \Sigma_{j=1}^{5}\Sigma_{j<i}\theta_{ij}u_j/\Sigma_{j=1}^{5}\Sigma_{j\neq i}\theta_{ij}u_j$ and u_j is the probability that an individual is found in site j after migration. Because $P(\text{from north}|\boldsymbol{\Theta})$ is a function of $\boldsymbol{\Theta}$, its posterior distribution may be calculated from the posterior samples obtained above using Gibbs sampling. The probability of having moved a given distance may be calculated similarly, assuming that movement is restricted to the sites sampled. For illustration purposes, we assume that individuals are uniformly distributed after migration, though this can certainly be improved upon with additional information on relative abundances in each location.

The sampling algorithm described above was implemented in Matlab version 6.5 (The Mathworks, Natick, MA 01760-2098, USA). Convergence was assessed using the Gelman–Rubin diagnostic for five chains with highly dispersed initial values (Gelman and Rubin 1992). For all samples tested, apparent convergence under the Gelman–Rubin diagnostic was attained with chains of 1000 samples. The results presented here are based on 5000 samples retained after discarding the first 5000 draws. Autocorrelation within chains was negligible (<0.1) at all lags, so the resulting chains were not thinned. The code is available from S. Munch upon request.

Application to weakfish

As an illustration of the Bayesian approach described above, we re-analyze otolith microchemistry data for weakfish. Weakfish are a marine fish species found along the eastern coast of the United States. Using otolith chemistry, Thorrold et al. (2001) investigated whether weakfish home to natal estuaries to spawn. Here we briefly sketch the sampling and results; for full details, refer to Thorrold et al. (1998, 2001). Juveniles were collected from coastal Georgia (GA), Pamlico Sound, North Carolina (NC), Chesapeake Bay (CB), Delaware Bay (DE), and Peconic Bay, New York (NY). Based on otolith chemistry, a classification rule was determined using linear discriminant analysis (Thorrold et al. 1998). Two years later, mature weakfish from the same year class were collected, and the juvenile classification rule was used to determine the site of origin for each adult fish in the mixed sample.

To evaluate the performance of the method for different sample sizes and sample compositions, we also conducted a small simulation study based on the weakfish data. For each sample size and composition combination, 100 artificial data sets were drawn by sampling with replacement from the DE and NY weakfish data. Sample sizes were the same for both training and sample data sets and ranged from 10 to 100 individuals. Mixture proportions ranged from 0.5 to 1. We evaluated how the posterior uncertainty and degree of bias varied with sample size and mixing proportions. For com-

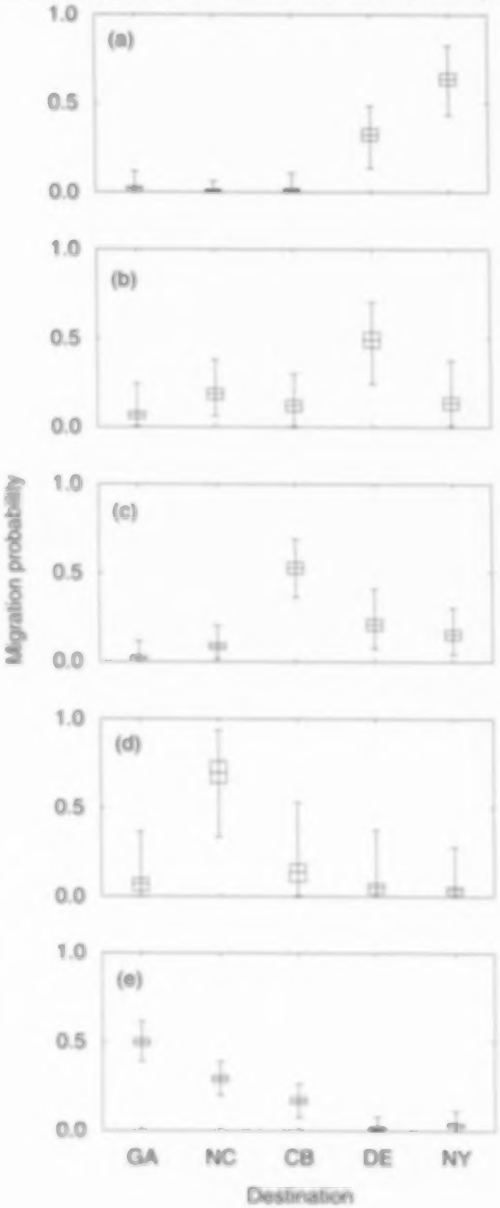

Fig. 3. Migration kernels by location of origin. (*a*) Summary of the posterior probability of an individual moving from New York to the estuary indicated on the horizontal axis. Similarly, *b*–*e* represent migration probabilities for Delaware, Chesapeake Bay, North Carolina, and Georgia, respectively. These migration probabilities were calculated assuming that the distribution of individuals after migration is uniform and that migration outside this region is impossible. In the box-and-whisker plots, the center line for each box gives the location of the posterior mean, the top and bottom box edges give the upper and lower quartiles of the distribution, and the whiskers give the entire support for the posterior (i.e., the sampled range).

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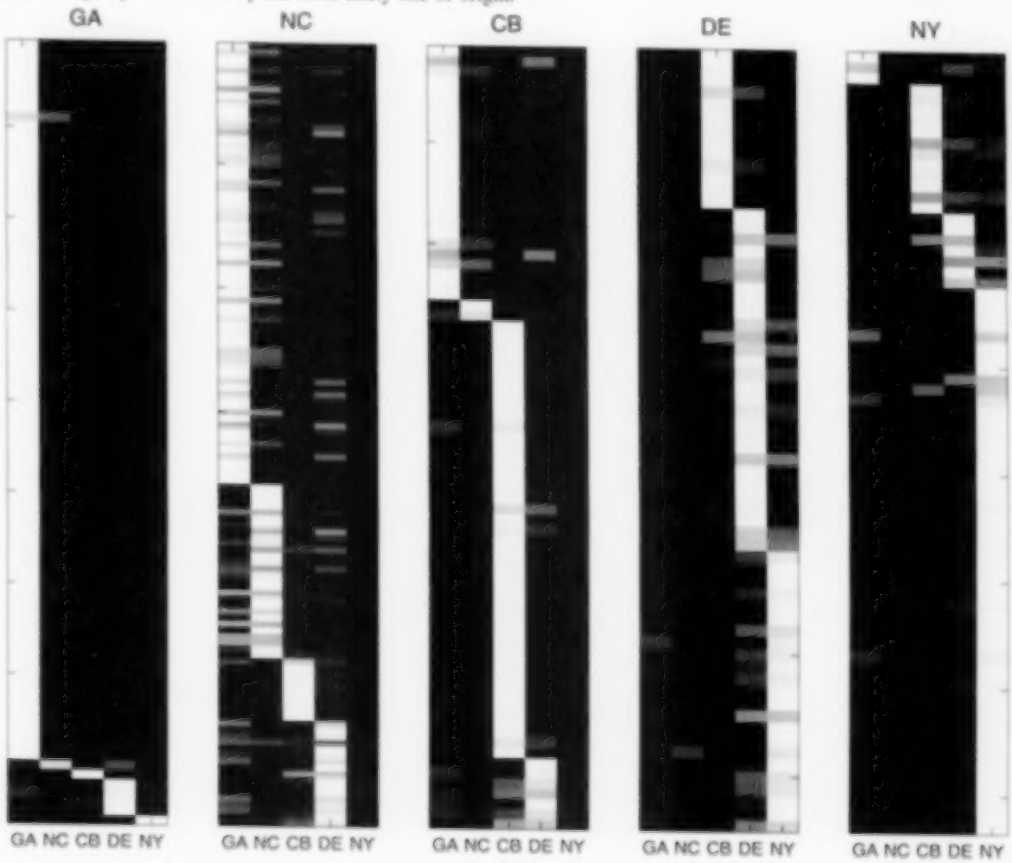
Fig. 4. Individual posterior classification probabilities. Each panel represents the classification results for all individuals collected at the estuary indicated on top. Within panels, the columns indicate putative sites of origin and the grey scale on each row represents an individual's probability of originating from each site. $P = 1$ is indicated in white and $P = 0$ is indicated in black. Rows that are black and white indicate well-classified individuals, rows that are grey depict individuals for which the site of origin is uncertain. The data for each estuary have been sorted to group individuals by the most likely site of origin.



parison, we also used the standard maximum likelihood approach based on the sample means and covariances from the training data (as in Millar 1990).

Results

Broadly speaking, we found that the spawning adults were frequently juveniles in the same location (Fig. 1). For example, the top-right panel of Fig. 1 illustrates that the posterior probability that fish captured in GA also originated in GA ranges from 0.7 to 0.95, with the posterior mode at 0.9. However, the posterior θ for fish captured in GA, but originating in NC, is less than 0.2. In general, the panels down the diagonal of this figure indicate that fish have the highest probability of originating from the site in which they were collected. This is true for each site with the exception of NC where the majority of spawners appeared to originate in GA. Although we cannot estimate the probability of homing without additional data on the population sizes in each location, these results do indicate support for the assertion that weakfish home. Moreover, we find evidence that straying is most likely to be among neighboring sites. That is, the greatest fraction of the nonresident spawning adults originated in adjacent sites rather than being drawn uniformly from the entire region.

This is seen more clearly in the migration estimates. The probability of migrating a given distance tails off approximately exponentially when averaged over locations (Fig. 2). Migration probabilities constructed for each estuary (Fig. 3) reveal some interesting asymmetries, particularly for Delaware and Chesapeake Bay. There is a modest north–south bias in the movement, with more migrants coming from the north. Specifically, the probability that migrants came from the north was on average 0.57, with a range of 0.55 to 0.59.

The individual posterior conditional assignment probabilities provide an estimate of the certainty with which individuals can be classified (Fig. 4). If the otolith signatures of the various populations showed complete separation, the assignment probabilities for each individuals would be 1 and 0. However, posterior conditional assignment probabilities indicate that there is considerable classification uncertainty for some individuals. Although aggregated posterior classifi-

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cation uncertainty was presented in Thorrold et al. (2001), the individual-specific posteriors shown here provide additional information about spatial structuring in the data. For instance, many of the individuals collected in North Carolina were classified to a site of origin with less than a probability of 1, indicating that the North Carolina signature may overlap substantially with other locations or that individuals collected in North Carolina represent heterogeneous signatures not well represented in the training data. In contrast, individuals collected in Georgia were classified with much greater certainty. Results also showed that most individuals classified with less than a probability of 1 were likely to be classified to the nearest estuaries. That is, when there is ambiguity in the origin of an individual, it is limited to neighboring sites rather than spread across the map.

Our simulation results indicate that for sample sizes greater than 30, the present method provides results that are quite similar to those of previous methods. As found in previous studies (see, e.g., Millar (1987) and Pella and Masuda (2001)), point estimates are biased when the mixture is very uneven, although the posterior mode is a less biased estimate of $\boldsymbol{\theta}$ than the posterior mean. The bias decreased with sample size and was less than 0.04 (6%) for sample sizes of 30 or more, regardless of the true mixing proportions. Both maximum likelihood and Bayesian estimates converge on the correct values as the sample size becomes large. Because we account for uncertainty in the training data, posterior uncertainty in $\boldsymbol{\theta}$ is greater for this method than the maximum likelihood approach, although this difference was never more than 10%.

Discussion

A Bayesian approach to addressing mixed-catch problems provides a straightforward means of dealing with uncertainty not recognized by other methods. Our method explicitly accounts for the small-sample uncertainty in the location-specific means and covariances that are used in constructing a classifier. Our comparison across methods indicated that accounting for this uncertainty is most important for extremely small sample sizes where maximum likelihood methods underestimated the posterior uncertainty by 10%. However, for samples sizes of about 30 individuals or more, the qualitative results appear to be robust to the choice of method, as both Bayesian and maximum likelihood methods yielded similar bias and posterior classification uncertainty. The chief advantage of the Bayesian framework is therefore the ease with which predictions can be made about ecologically relevant functions of the parameters.

In particular, we focused on obtaining a posterior distribution for the dispersal kernel and directional bias in migration. We found that movement was somewhat biased north to south, which is in keeping with the major coastal currents that migrating weakfish are likely to encounter. GA appears to be relatively isolated from northern migrants, and the sample from NC was the most heterogeneous. Again, this is in keeping with the dominant oceanographic features of the coast; at Cape Hatteras, the warm northbound Gulf Stream and the cold southbound Labrador Current meet, creating a mixing zone. Cape Hatteras, NC, is a well-known zoogeographic barrier.

Prior analyses have not examined in any detail the individual classification probabilities, although this information has, in principle, been available. White and Ruttenberg (2007) show that classification success is improved when, as done here, mixing proportions are estimated as part of the classification model and the importance of evaluating classification accuracy. Although all of the models used to date, including ours, assume that the misclassification probability is independent of the site of origin, we found that there was substantial spatial structuring in the posterior classification probabilities. This structuring creates some ambiguity in the interpretation of mixing fractions and derived migration rates. In particular, it becomes impossible to assess whether the observation that migrants are most likely from an adjacent site is due to migration or instead to neighboring sites having similar otolith chemistry. There are two approaches to dealing with this problem, one empirical and one statistical. An empirical solution to this problem would be to find otolith constituents with concentrations effectively independent across locations. Although this might be the most rigorous way to go, there is no obvious route to efficiently identifying which elemental signatures will be independent of location. A statistical approach would involve explicitly modeling the spatial dependence of the otolith signatures and inferring site-specific means and covariances that take into account this additional information. This route may also be promising for other reasons described below.

A substantial obstacle to the use of classification-based methods for inferring migration rates is the fact that they implicitly assume that all possible sites of origin are represented in the training data. For most marine species, this is a logistical impossibility. Although this logical flaw has been recognized by other authors (Campana et al. 2000; Manel et al. 2002), there has been no progress made in eliminating it. We see two potential routes to dealing with this problem, neither of which has been applied to date. The first approach is to set some threshold distance from the mean beyond which an individual cannot be classified. In the context of multivariate normal data, an obvious choice would be to use the fact that Mahalanobis distances follow the chi-square distribution (Krzanowski 1988) to set the threshold. Although practical, this approach relies on a frequentist argument for setting the threshold and is somewhat at odds with our Bayesian approach. An alternative would be to make use of the spatial dependence in the otolith signatures. That is, by asserting that the otolith chemistry varies with location in a relatively smooth way, we could, in principal, obtain a continuous posterior distribution for the location origin for each individual. This method might also reduce the bias in the estimated mixing proportions as described above. We are currently working on developing such a method based on Gaussian processes.

As in any Bayesian analysis, some comment on prior specification seems warranted. We have used flat or conjugate priors that are close, if not identical, to Jeffreys priors. These were chosen to represent minimal prior information. However, there may be cases where additional prior information is available. For instance, it may be possible to obtain priors on the mixing fractions from tagging studies or oceanographic data. If, for example, previous research had indicated that the dispersal kernel was approximately exponential with decay rate ϕ, we could choose $\boldsymbol{\alpha}$ such that the prior mean for θ_{ij} is equal to $ae^{-\phi d_{ij}}$, where d_{ij} is the distance between sites i and j and a is a scalar that controls the prior

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uncertainty in dispersal. Although this sort of prior is probably too informative when the goal of the study is to learn about mixing, it would be quite useful when the goal is to classify a set of individuals. It is also possible to envision obtaining prior information on the mean otolith signatures from water chemistry or previous otolith studies. For instance, we might expect that otoliths from water bodies near major urban centers would have higher concentrations of Pb or Cu or that areas near major freshwater inputs would have lower Sr and higher Ba values. Additionally, oxygen isotope signatures may vary with latitude. When available, this sort of information may be used to help construct informative priors.

Given the variability in otolith data, it may be worthwhile to evaluate the role of measurement error in determining classification success. Currently used methods either assume that measurement errors are zero or implicitly confound variability and measurement error. Although uncertainty in the measurement of the chemical signatures is likely to be small relative to the variability among individuals within sites, there may be some value in explicitly representing these errors, particularly when different protocols are used for the sample and training data. If, for example, the training and sample data are averages of different numbers of observations for each otolith, then the differences in measurement uncertainty ought to be accounted for. Doing so is difficult in traditional discriminant analyses but relatively straightforward in the Bayesian setting using hierarchical or mixed-effects models (Clark (2007) provides an excellent introduction). As an example, if measurement errors are additive and multivariate normal with mean zero and covariance $\mathbf{U}$, then the mean for each otolith will be normally distributed with mean $\boldsymbol{\mu}_i$ and covariance $\boldsymbol{\Sigma}_i + \mathbf{U}n^{-1}$ and this inflated covariance should be used in place of $\boldsymbol{\Sigma}$. This construction will be particularly useful when some estimate of $\mathbf{U}$ is available.

Our model, along with all discriminant function and maximum likelihood-based otolith analyses, assumes that otolith signatures follow a multivariate normal distribution on some scale of measurement. Because the normality assumption is difficult to rigourously justify, it may be worthwhile to contemplate alternative approaches. Finite mixture models in which the likelihood is represented by a weighted sum of several densities provide increased flexibility (for an analgous approach on genetic data, see Bolker et al. 2007). Distributional assumptions may be relaxed almost entirely by moving to nonparametric Bayesian methods, such as those based on Dirchlet process models (Ferguson 1973; Antoniak 1974). Machine learning methods such as neural networks and support vector machines do not make explicit distributional assumptions and have been useful in previous otolith studies (Thorrold et al. 1998; Castro 2007). However, because these methods are not explicitly probabilistic, it is difficult to construct Bayesian methods for them.

The Bayesian approach that we described provides a straightforward means of estimating mixing proportions and associated uncertainty. It also provides simple means of visualizing spatial structure in the classification probabilities and migration patterns. Incorporation of these two factors into the mixed-stock composition estimation approach highlights valuable avenues for further method development. This method builds on previously existing methods for genetic data (Pella and Masuda 2001) and provides an approach that is suitable for the growing number of otolith studies. As the use of otolith chemistry as a tool for estimating population structure continues to grow, an appropriate approach for estimating the mixed-stock composition becomes increasingly important. The method proposed here is appropriate for any continuous multivariate data that is normally distributed on some scale of measurement and should prove useful for making ecological inferences from otolith chemistry data.

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Appendix A

The posterior distribution $p(\boldsymbol{\mu}_s, \boldsymbol{\Sigma}_s|\mathbf{X}_s)$ for each of the site-specific means and covariance matrices is normal-inverse Wishart (Gelman et al. 1995). It is most conveniently written in the hierarchical form $p(\boldsymbol{\mu}_s|\boldsymbol{\Sigma}_s, \mathbf{X}_s)p(\boldsymbol{\Sigma}_s|\mathbf{X}_s)$ where

$$p(\boldsymbol{\mu}_s|\boldsymbol{\Sigma}_s, \mathbf{X}_s) = \left(\frac{N_s}{2\pi}\right)^{q/2} |\boldsymbol{\Sigma}_s|^{1/2} e^{-\frac{N_s}{2}(\bar{X}_s - \boldsymbol{\mu}_s)^T \boldsymbol{\Sigma}_s^{-1}(\bar{X}_s - \boldsymbol{\mu}_s)}$$

$$p(\boldsymbol{\Sigma}_s|\mathbf{X}_s) = \frac{|(N_s - 1)\hat{\mathbf{C}}_s + \mathbf{W}|^{(N_s+v-1)/2}}{2^{(N_s+v-1)q/2}\Gamma_q\left(\frac{N_s+v-1}{2}\right)|\boldsymbol{\Sigma}_s|^{(N_s+v+q)/2}} e^{-\frac{1}{2}tr\{[(N_s-1)\hat{\mathbf{C}}_s + \mathbf{W}]\boldsymbol{\Sigma}_s^{-1}\}} \quad \text{(A1)}$$

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where $\bar{\mathbf{x}}_s$ and $\hat{\mathbf{C}}_s$ are the sampled mean and covariance for the training data from site s.

To classify the sample data, we need the likelihood of obtaining each new observation $\mathbf{y}_i$ given that it came from a specific site σ_i. This is given by the posterior predictive distribution $p(\mathbf{y}_i|\sigma_i = s, \mathbf{X}_s)$, which is obtained by integrating over the mean and covariance

$$\text{(A2)} \quad p(\mathbf{y}_i|\sigma_i = s, \mathbf{X}_s) = \int\int p(\mathbf{y}_i|\sigma_i = s, \boldsymbol{\mu}_s, \boldsymbol{\Sigma}_s)p(\boldsymbol{\mu}_s, \boldsymbol{\Sigma}_s|\mathbf{X}_s)\mathrm{d}\boldsymbol{\mu}_s\mathrm{d}\boldsymbol{\Sigma}_s$$

Evaluating this integral, we obtain the posterior predictive distribution given in eq. 4. Briefly, this is accomplished by completing the square (as in Hilborn and Mangel 1997) and integrating over the resulting normal distribution for the mean. The remaining terms that contain the covariance matrix may be integrated out by analogy with the Wishart distribution.

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Stream trees: a statistical method for mapping genetic differences between populations of freshwater organisms to the sections of streams that connect them

Steven T. Kalinowski, Michael H. Meeuwig, Shawn R. Narum, and Mark L. Taper

Abstract: Statistical approaches for studying the spatial distribution of genetic diversity that assume that organisms move through a two-dimensional landscape are not well suited to study populations of freshwater fish. We present a new statistical method for mapping genetic differences among populations of freshwater fish to the sections of streams that connect them. The method is useful for freshwater species that can only disperse through stream corridors and for other species that live in habitats for which there is one, and only one, corridor connecting each pair of populations (e.g., alpine organisms confined to ridge tops). The model is a simple extension of the least-squares method for constructing evolutionary trees. In this model, the genetic distances between populations are modeled as a sum of genetic distances mapped onto landscape features (e.g., stream sections). Analysis of simulated data shows that the method produces useful results with realistic amounts of data. The model was fit to empirical microsatellite data from four metapopulations of freshwater fish and showed an excellent fit in three out of four cases. Software to perform the necessary calculations is available from the authors at www.montana.edu/kalinowski.

Résumé : Les méthodologies statistiques pour l'étude la répartition spatiale de la diversité génétique qui présupposent que les organismes se déplacent dans un paysage bidimensionnel ne conviennent pas bien à l'étude démographique des poissons d'eau douce. Nous présentons une nouvelle méthode statistique pour cartographier les différences génétiques entre des populations de poissons d'eau douce en relation avec les cours d'eau qui les relient. Cette méthode est utile pour les espèces d'eau douce qui ne peuvent se disperser que par des corridors formés par des cours d'eau et pour les autres espèces pour lesquelles il existe un, et un seul, corridor reliant chaque paire de populations (par ex., les organismes alpins confinés aux sommets des crêtes). Le modèle est une simple extension de la méthode des moindres carrés utilisée pour construire des arbres phylogénétiques. Dans ce modèle, les distances génétiques entre les populations sont représentées comme la somme des distances génétiques cartographiées sur des éléments du paysage (par ex., des sections de cours d'eau). L'analyse de données simulées montre que la méthode fournit des résultats utiles avec un nombre réaliste de données. Nous avons ajusté le modèle à des données empiriques sur les microsatellites dans quatre métapopulations de poissons d'eau douce et obtenu un excellent ajustement dans trois des quatre cas. On peut obtenir des auteurs le logiciel pour réaliser les calculs nécessaires à www.montana.edu/kalinowski.

[Traduit par la Rédaction]

Introduction

The goal of landscape genetics is to understand how geography shapes the genetic composition of populations (e.g., Manel et al. 2003; Storfer et al. 2007). The first step in accomplishing this is usually to describe the spatial distribution of genetic variation. Once genetic relationships among populations have been mapped onto the physical landscape, the influence of landscape features upon genetic structure may be inferred. Increasingly abundant genetic data have recently heightened interest in such research, but investigations of genetic diversity and geography date back many decades (e.g., Wright 1943; Edwards and Cavalli-Sforza 1964).

Traditional methods for describing genetic differences between populations often have disadvantages that limit their usefulness for answering spatial questions. For example, the most commonly used strategy for inferring how geography has influenced population structure is to construct an evolutionary tree showing genetic relationships between populations and then use this tree to identify landscape features that are associated with genetic discontinuities. This approach can

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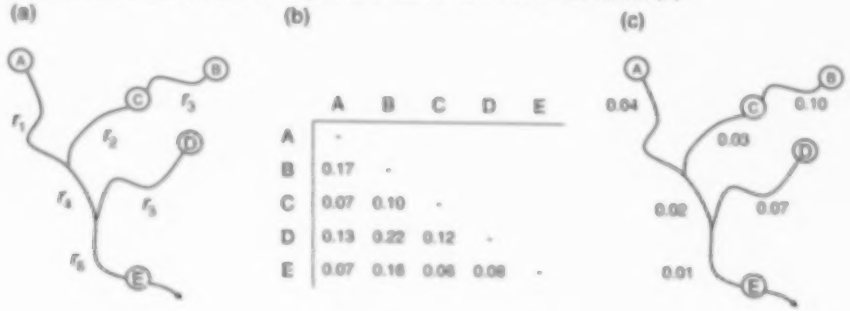
Fig. 1. (*a*) A map showing the locations of five hypothetical populations of fish (A–E) connected by six stream sections. (*b*) A matrix of genetic distances for the five populations. The goal of the statistical method described in this manuscript is to estimate the parameters r_1 to r_6 so that the sum of the rs between each pair of populations is equal (or nearly equal) to the genetic distance observed between them. Such estimates are shown in the "stream tree" in *c*. In this example, there is a perfect fit of the model to the data (i.e., the sum of the genetic distances in the stream tree (*c*) are exactly equal to the genetic distances observed in the matrix (*b*).



be very successful. For example, a tree showing genetic relationships among populations of Atlantic salmon (*Salmo salar*) clearly showed that the largest genetic differences were associated with the Atlantic Ocean (King et al. 2001). Most trees, however, are not so easy to interpret because trees show only genetic similarity; relating the branching pattern of a tree to the physical landscape is often difficult.

The relationship between population genetic structure and geography is often explored by testing for an isolation-by-distance relationship. A significant correlation between genetic difference and geographic distance means that genetic differences are proportional to geographic distances and suggests that the rate of gene flow between populations is proportional to how far they are apart. If genetic differentiation shows a strong isolation-by-distance pattern, no further analysis may be required to explore how geography and genetic structure are related. However, the absence of correlation between genetic differentiation and geographic distance must be interpreted with caution, because there are two very different explanations for how such population structure could evolve. There may be no correlation between genetic structure and geography either because geography has not played an evolutionarily important role in shaping genetic population structure or because other geographic variables besides distance have been important. For example, barriers may prevent gene flow between populations that are close to each other, and corridors may facilitate gene flow between populations that are far apart. If a landscape contains barriers and corridors, populations on the landscape may not show an isolation-by-distance pattern, even though the genetic structure of the populations may have been shaped entirely by the physical landscape.

Many exciting analytic methods have recently been developed to map genetic differences among individuals and populations (e.g., Manni et al. 2004; Miller 2005; Foll and Gaggiotti 2006). The relative merits of these methods and related techniques have not been clearly identified, but most of these methods assume that individuals move through a two-dimensional landscape. For many freshwater organisms, there is only one potential corridor between each pair of populations, and this information should be taken into account when studying how genetic diversity is distributed throughout the landscape. Most of the current statistical approaches for studying landscape genetics cannot do this (the computer program BARRIER, described by Manni et al. (2004), is an exception) and are, therefore, poorly suited for describing genetic diversity for many freshwater fishes.

In this paper, we present a simple statistical method for mapping genetic differences between populations to the sections of streams that connect them. The method is a slight modification of the least-squares approach for making evolutionary trees and therefore has the advantage of using established statistical methods that have proven to be useful. We present this tool as a method for partitioning genetic differences among populations living in a watershed, but the method could be used for any populations for which there is only one possible path between each pair of populations (e.g., alpine populations connected by ridges, coastal organisms living on a shoreline).

A spatial model of genetic differentiation

Let us assume that a matrix of pairwise genetic distances (e.g., F_{ST}) has been estimated for a set of populations connected by streams in a watershed. The goal of our analysis is to map the genetic differences between these populations to the streams that connect them. The model that we propose does this by assigning each section of stream in the watershed a genetic distance that quantifies how much genetic differentiation occurs across that stream section. If, for example, a stream section contains a waterfall that is a barrier to gene flow, we would probably expect that stream section to be assigned a large genetic distance. Let us represent the genetic distance mapped to the kth stream section as r_k (Fig. 1). The algorithm that we describe below assigns values of r to each stream section in such a manner that if we sum up the rs for all of the stream sections between a pair of populations, the sum of these genetic distances will equal the observed genetic distances between the populations. For example, if the pairwise F_{ST} between populations A and B is equal to 0.17, and populations A and B are separated by stream sections 1, 2, and 3, we seek values of r_1, r_2, and r_3 so that $r_1 + r_2 + r_3 = 0.17$ (Fig. 1).

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The main assumption in our model is that genetic distances between populations can be modeled as a sum of genetic distances for the stream sections that connect them. This may seem to be an overly simplistic model of genetic differentiation, but it makes the same assumption used to construct additive evolutionary trees (e.g., neighbor joining, unweighted pair group method with arithmetic mean (UPGMA); Felsenstein 2004). Additive evolutionary trees are constructed so that the sum of branch lengths connecting each pair of populations is approximately equal to the observed genetic distance between the populations. The main difference between the stream-based approach that we describe below and an evolutionary tree is that we use the network of streams in the watershed as the topology of the tree, with each stream section in the watershed corresponding to a branch in an evolutionary tree. For this reason, we call our model of genetic differentiation a stream tree.

Stream trees can be constructed from a matrix of genetic distances as follows. Let N represent the number of populations that have been sampled in a watershed, and let D_{ij} represent the genetic distance observed between populations i and j. Let S represent the total number of stream sections in the watershed (see below for a discussion of exactly how to define stream sections). Let r_k represent the genetic distance mapped to the kth stream section (Fig. 1), and let $\mathbf{r}$ represent the vector of all these distances, $\mathbf{r} = [r_1, r_2, \ldots, r_S]$. We seek values of $\mathbf{r}$ so that the sums of the genetic distances for stream sections connecting populations are close to the observed genetic distances between the populations. We use the indicator variable $x_{ij,k}$ to calculate these sums. $x_{ij,k}$ equals one if stream section k is between populations i and j and equals zero otherwise. The genetic distance predicted by our model between populations i and j, d_{ij}, is equal to

$$(1) \qquad d_{ij} = r_1 x_{ij,1} + r_2 x_{ij,2} + \ldots + r_S x_{ij,S}$$

We use least-squares estimation to estimate $\mathbf{r}$ (Cavalli-Sforza and Edwards 1967; for a review, see Felsenstein 2004, chapter 11). Let Q represent the sum of squared discrepancies between predicted genetic distances and observed genetic distances.

$$(2) \qquad Q = \sum_{i=1}^{N} \sum_{j=i+1}^{N} (D_{ij} - d_{ij})^2$$

Least-squares estimates of $\mathbf{r}$ are the values of $\mathbf{r}$ that minimize Q. Several iterative and exact methods have been developed to find values of $\mathbf{r}$ for the mathematically identical problem of phylogeny estimation. Here we use a slight modification of Cavalli-Sforza and Edwards' (1967) approach. To estimate $\mathbf{r}$, we construct a matrix of genetic distances, $\mathbf{D}$, and a matrix of indicator variables, $\mathbf{X}$. The genetic distance matrix $\mathbf{D}$ contains the genetic distances for the N sampled populations arranged in a single column with $N(N - 1)/2$ rows. We order the genetic distances so that the first row contains the genetic distance for populations 1 and 2, the second row contains the genetic distance for populations 1 and 3, and so forth. In the example shown in Fig. 1, $\mathbf{D}$ equals

$$(3) \qquad \mathbf{D} = \begin{bmatrix} D_{AB} \\ D_{AC} \\ D_{AD} \\ D_{AE} \\ D_{BC} \\ D_{BD} \\ D_{BE} \\ D_{CD} \\ D_{CE} \\ D_{DE} \end{bmatrix}$$

The matrix $\mathbf{X}$ contains the coefficients $x_{ij,k}$ in a matrix with $N(N - 1)/2$ rows and S columns. Each row corresponds to a comparison between two populations (arranged in the same order as $\mathbf{D}$), and each column corresponds to a stream section. In the example shown (Fig. 1), $\mathbf{X}$ equals

$$(4) \qquad \mathbf{X} = \begin{bmatrix} 1 & 1 & 1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 1 & 1 & 0 \\ 1 & 0 & 0 & 1 & 0 & 1 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 1 & 1 & 1 & 1 & 0 \\ 0 & 1 & 1 & 1 & 0 & 1 \\ 0 & 1 & 0 & 1 & 1 & 0 \\ 0 & 1 & 0 & 1 & 0 & 1 \\ 0 & 0 & 0 & 0 & 1 & 1 \end{bmatrix}$$

As above, a one in the matrix indicates that a stream section connects two populations (or is at least part of the connection between populations). Given these matrices, least-squares estimates of $\mathbf{r}$ are obtained

$$(5) \qquad \mathbf{r} = (\mathbf{X}^T\mathbf{X})^{-1}\mathbf{X}^T\mathbf{D}$$

(Cavalli-Sforza and Edwards 1967). This method for estimating the genetic distances of each stream section in our model has been used for a long time to estimate branch lengths in evolutionary trees, e.g., by the computer program FITCH (part of the PHYLIP software package; Felsenstein 2005). The neighbor-joining algorithm for estimating trees (Saitou and Nei 1987) has probably been used more often than least-squares approaches, but both approaches are mathematically related (Felsenstein 2004).

A well-known problem with eq. 5 is that some of the estimated branch lengths can be negative (Felsenstein 1997), and negative genetic distances have no biological meaning. We have used the following iterative procedure when one or more estimates are negative. First, we identify the genetic distance that is most negative and constrain it to equal zero. This is done by removing its column from matrix $\mathbf{X}$. Then we re-estimate the genetic distances for the remaining stream sections. If some of resulting estimates are negative, we constrain the most negative distance to equal zero (in addition to the distance previously constrained to equal zero) and continue in this fashion until all estimates are nonnegative. Algorithms like this are commonly used in constrained optimizations (McCulloch and Searle 2001).

Once estimates of $\mathbf{r}$ have been obtained, it is natural to ask if the resulting model fits the data well. This can be

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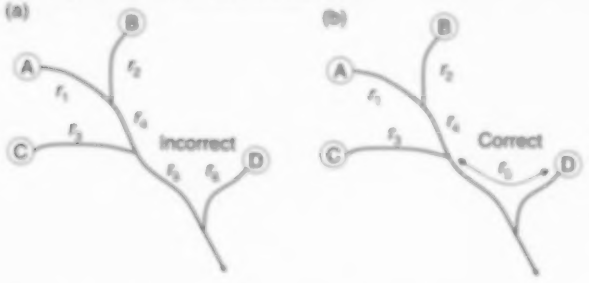
Fig. 2. A hypothetical stream network having four sampled populations (A–D) and stream sections defined either (*a*) incorrectly or (*b*) correctly for the stream network model.



done by calculating the coefficient of determination, R^2, for the stream tree:

$$(6) \qquad R^2 = 1 - \frac{\sum_{ij}(d_{ij} - D_{ij})^2}{\sum(D_{ij} - \bar{D})^2}$$

where $\bar{D}$ is the average pairwise genetic distance between the populations and summation is taken over all pairs of populations. If R^2 is nearly equal to 1.0, the stream tree provides a good fit to the observed data.

We have modeled the genetic distance between each pair of populations as a linear function of the genetic distances for the stream sections connecting them (eq. 1) and have used least-squares estimation to estimate these stream-specific distances (eq. 5). This approach is mathematically equivalent to linear regression through the origin, which means that any linear regression software package can be used to estimate $\mathbf{r}$. If regression is used, the observed pairwise genetic distance between populations is the dependent variable. The indicator variables x_{ijA} are the independent variables, and the branch-specific genetic distances $\mathbf{r}$ are the unknown parameters in the regression model. This is a no-intercept model. Appendix A (Table A1) illustrates how the data might be organized to perform this analysis. There are two computational advantages to this approach. First, it can be implemented with ordinary statistical software. Second, some software packages offer methods for constraining regression coefficients to be positive, so the iterative approach described above may not need to be used. To prevent confusion, we note that some software packages calculate R^2 from uncorrected sum of squares for a no-intercept model instead of as we have in eq. 6.

Now that we have described the general structure of our geographic model of population differentiation and explained how the model parameters can be estimated, we will clarify exactly how to define stream sections. Each sampled population must first be labeled on a map. Next, the minimum number of stream sections connecting the populations must be identified. Each of these stream sections must end at either a sampled population or an intersection with *two* other stream sections. Stream sections may not be defined so that they are arranged in tandem fashion in which one section starts at the end of another (unless there is a sampled population at that point). A potentially confusing scenario occurs when a tributary enters a main-stem section downstream of all other populations (population D in Fig. 2). The tributary and the main-stem section immediately upstream of the tributary must be considered as a single stream section. This is because all fish passing through the tributary (e.g., coming from or going to population D in Fig. 2) must also pass through the adjacent section of the main stem if they are to travel to another population. There is no way to differentiate between an obstacle to movement in the tributary or the adjacent section of the main stem. In mathematical terms, if the main-stem section and the tributary were treated independently (e.g., Fig. 2*a*), there would not be a unique solution to eq. 2. Any pair of genetic distances for the tributary and immediately upstream main-stem section (r_5 and r_6 in Fig. 2) that had the same sum would fit the observed data equally well. Therefore, for the model to be applied correctly, the sections must be labeled as a single section (r_5 in Fig. 2*b*).

Materials and methods

Simulations

We used computer simulation to test whether the stream tree algorithm produced reasonable population structures using realistic amounts of data. These tests were not intended to be comprehensive; instead, we attempted to verify that the method worked well in circumstances that it should and that it clearly failed when applied to populations with inappropriate evolutionary histories. In each test, genotypes were simulated for samples from populations in a watershed, and the stream tree algorithm was used to estimate genetic distances for each stream section in the watershed. Three spatial models of gene flow were used in the simulations: a headwater model, a linear stepping stone model, and a semi-realistic model having a complex model of gene flow.

In the headwater model of gene flow (Fig. 3), eight populations were located at the headwaters of a watershed. We assumed that the rate of gene flow between populations was proportional to how closely populations were connected in the watershed. Populations that were connected through the upper part of the watershed (e.g., populations A and B) were given a migration rate of 10^{-1}. Populations that had connections in the middle and lower parts of the watershed were given pairwise migration rates of 10^{-3} and 10^{-5}, respectively. Each population was assumed to have a constant effective population size (N_e) of 1000. The infinite alleles mutation rate used to simulate genotypes was equal to 10^{-4}. Genotypes were simulated for 12 unlinked loci using standard coalescent methods (Hudson 1990). F_{ST} (Weir and Cockerham 1984) was used as a pairwise genetic distance.

Two variations of the headwater model of gene flow were also examined. In the "variable N_e" simulation, half of the populations (B, D, F, and H; Fig. 3) in the watershed were given a N_e of 500, and half the populations (A, C, E, and G) were given an N_e of 1000. Genetic drift is stronger in smaller populations, so in this scenario, the stream sections that connect these smaller populations (sections 2, 4, 6, and 8; Fig. 3) to the rest of the watershed should have a larger genetic distances than the stream sections connecting to the larger populations (sections 1, 3, 5, and 7). A second variation of the headwater model examined the impact of headwater exchange on the ability of a stream tree to describe genetic structure. In this model, genotypes were simulated using migration rates as above. However, before a stream

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Fig. 3. The headwater model of gene flow used in simulations.

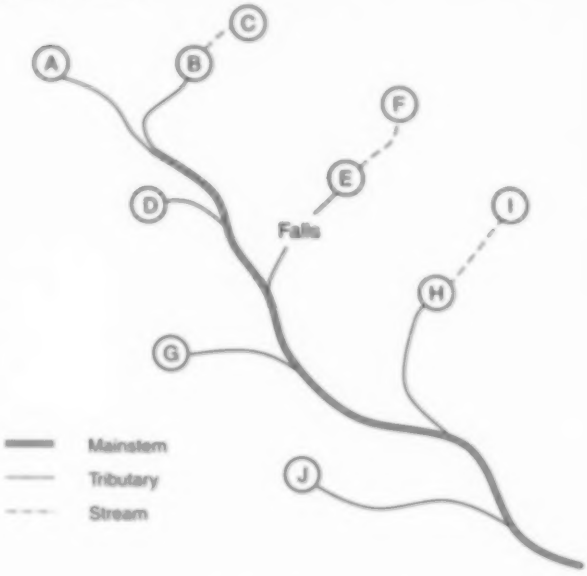
Fig. 4. The linear stepping stone model of gene flow used in simulations. Each population (A–H) was assumed to have the same effective population size and the same rate of gene flow into adjacent populations.

tree was constructed for the watershed, the samples from populations D and E were thoroughly admixed.

In the linear stepping stone model of gene flow (Fig. 4), the rate of gene flow between adjacent populations was assumed to be equal and symmetric. Three different migration rates were examined: m = 0.01, 0.003, and 0.001. N_e for each population was 1000. As for all models, the infinite alleles mutation rate was 10^{-4}. Genotypes were simulated for 12 loci.

The third and last spatial model used to validate the stream tree algorithm was the semi-realistic model. This model assumed that rate of gene flow between populations was determined by the geography of the watershed. The model was inspired by an ongoing study of bull trout (*Salvelinus confluentus*) population genetic structure in Glacier National Park, Montana (M.H. Meeuwig, unpublished data). Four types of waterways were modeled: a main-stem river, tributary rivers, streams within tributaries, and a waterfall (see Fig. 5 for a map depicting the hypothetical watershed). Each of these four types of river sections was assigned a barrier coefficient, z, that quantified how much of a barrier to gene flow the section represented. The migration rate between each pair of populations was then modeled as $10^{-\Sigma z}$, where summation was taken over the barrier coefficients for each stream section between the populations. The barrier coefficient for main-stem river sections was given a value of 0.0, indicating that it was not a barrier to gene flow. Streams were assigned a barrier coefficient of 1.0, tributaries a coefficient of 2.0, and the waterfall a coefficient of 4.0 (indicating that the waterfall reduced the rate of gene flow by a factor of 10^{-4}). For simplicity, migration rates were assumed to be equal upstream and downstream. A few examples illustrate how migration rates were calculated (Fig. 5). The migration rate between populations A and B was $10^{-(1+1)} = 0.01$. The migration rate between populations D and E was $10^{-(1+1+1+1)} = 0.00001$. As above, N_e for each population was assumed to equal 1000, and the infinite alleles mutation rate

Fig. 5. The semi-realistic model of gene flow used in computer simulation. Each type of waterway in the figure (main stem, tributary, stream, and waterfall) was assumed to affect gene flow in a different manner.



was assumed to equal 10^{-4}. Genotypes were simulated for data sets with 12 loci.

Empirical examples

We constructed stream trees of four metapopulations of trout to illustrate the method and to assess whether stream trees fit empirical data well. The four data sets that we analyzed included (*i*) 19 populations of rainbow trout (*Oncorhynchus mykiss*) in the Klickitat River basin, Washington, genotyped at 13 microsatellite loci (Narum et al. 2008), (*ii*) 19 populations of bull trout in Glacier National Park, Montana, genotyped at 10 microsatellite loci (M.H. Meeuwig, unpublished data), (*iii*) 20 populations of bull trout in the Boise River, Idaho, genotyped at six microsatellite loci (Whiteley et al. 2006), and (*iv*) 16 populations of Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*) in the Marys River drainage, Nevada, genotyped at 11 microsatellite loci (Neville et al. 2006). We used F_{ST} (Weir and Cockerham 1984) as a pairwise genetic distance and used the statistical software package SAS (SAS version 9.1; SAS Institute Inc., Cary, North Carolina) to construct stream trees and calculate R^2 values (eq. 6). We were interested in how stream trees would compare with a traditional bifurcating evolutionary tree, so we made a neighbor-joining tree (Saitou and Nei 1987) for each of these four data sets and calculated a R^2 value for the tree in the same way as we did for stream trees.

Results

The stream tree algorithm did a very good job of describing genetic structure in the simulated models of gene flow. Results for each of the three models will be presented in turn.

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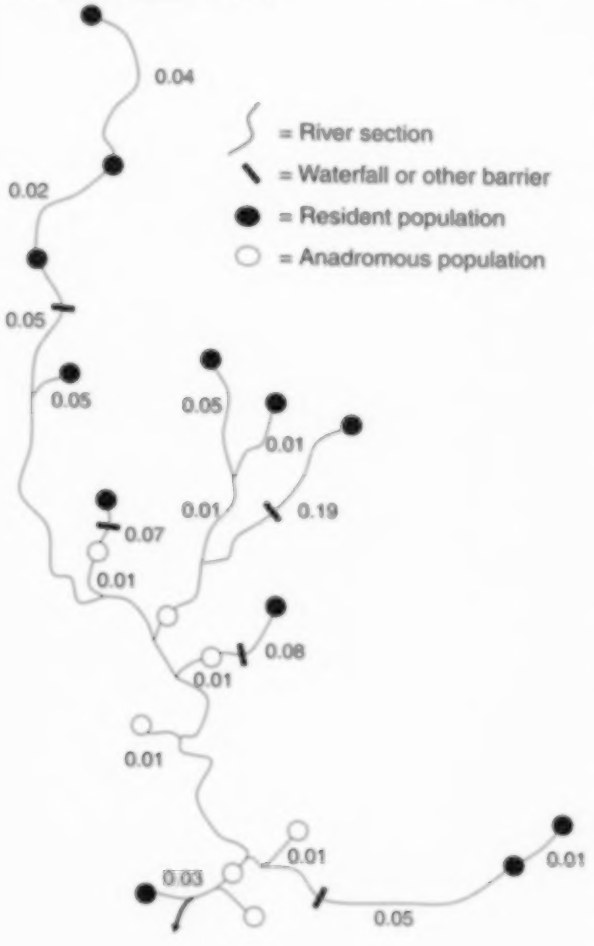
Table 1. The proportion of variation explained by the stream tree model of genetic differentiation described in this paper and neighbor-joining (NJ) trees for four metapopulations of freshwater fish.

Study	Species	N	S	F_{ST}	R^2	
					Stream tree	NJ tree
Narum et al. 2008	Rainbow trout	19	28	0.10	0.974	0.987
M.H. Meeuwig, unpublished data	Bull trout	19	29	0.27	0.971	0.952
Neville et al. 2006	Cutthroat trout	16	22	0.12	0.994	0.934
Whiteley et al. 2006	Bull trout	20	36	0.06	0.681	0.742

Note: N, number of populations sampled; S, number of stream sections in the watershed; F_{ST}, global F_{ST} for all populations sampled. Rainbow trout, *Oncorhynchus mykiss*; bull trout, *Salvelinus confluentus*; cutthroat trout, *Oncorhynchus clarkii henshawi*.

Fig. 6. The stream tree for rainbow trout (*Oncorhynchus mykiss*) populations in the Klickitat River. All sections of river not labeled have a genetic distance of zero.



The average R^2 value for the headwaters model was greater than 0.99, showing that the stream tree did an excellent job of fitting the model to the data. The results also made evolutionary sense. The average genetic distance assigned to the stream sections in the headwaters, which were connected by high rates of gene flow, was 0.0022; the average genetic distance assigned to middle reaches, which had a lower rate of gene flow, was 0.012; and the average genetic distance assigned to the lower reaches, which had the lowest rate of gene flow, was 0.13.

Results were similar for the variable N_e version of the headwaters model. The average R^2 was greater than 0.99, and the average genetic distance for the headwater streams connected to populations with an N_e of 500 (stream sections 2, 4, 6, and 8) was nearly twice as large as the genetic distance mapped to headwater streams connected to populations with an N_e of 1000 (stream sections 1, 3, 5, and 7) (0.0046 vs. 0.0025). This shows that the stream tree algorithm was successfully able to identify the genetic differentiation associated with the stronger genetic drift in the small populations.

As expected, the stream tree algorithm did not do a good job of describing population structure for the headwater exchange model. In this model, populations D and E were genetically identical, despite being far apart on the network of streams. The average R^2 for this model was 0.72 (down from 0.99 for the standard headwater model). This shows that violations of the assumption of the stream tree model of genetic differentiation can be detected by checking R^2 values.

The stream tree algorithm generally did a good job of describing population structure in the linear stepping stone model, but the simulations revealed an important limitation of the stream tree approach. The R^2 value for stream trees constructed for the linear stepping stone model was 0.94 when the migration rate was 0.01 between adjacent populations. This R^2 is lower than values obtained for the headwater model reported above. The decrease is apparently caused by sampling error, for if the number of loci sampled were increased, R^2 would climb towards 1.0 (results not shown). Not only did stream trees constructed from linear stepping stones have relatively high R^2 values, but the genetic distances assigned to the stream sections faithfully depicted the symmetric pattern of gene flow between populations. For the case of a migration rate of 0.01, each stream section was assigned a genetic distance of approximately 0.011. When the migration rate was decreased to 0.003, the fitted genetic distance between populations increased to approximately 0.031, and the R^2 value stayed high (average = 0.93).

Something interesting happened, however, when stream trees were made for the stepping stone model having a lower rate of gene flow. When the migration rate was decreased to 0.001, the average R^2 value dropped to 0.74 (and even lower values were obtained when lower rates of gene

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flow were tested). This relatively poor fit was not caused by sampling error but by an inability of the stream tree to accurately depict genetic relationships among populations that are highly differentiated. A close look at the stream tree illustrates the problem. When the rate of gene flow between adjacent populations was 0.001, the average genetic distance between adjacent populations was approximately 0.10. The stream tree algorithm might be expected, therefore, to assign a genetic distance of 0.10 to each stream section in the linear stepping stone model. However, if this was done, the genetic distance between the first and last of the eight populations would be equal to 0.7. This would be a problem, because the maximum value of F_{ST} is approximately equal to the average homozygosity of the loci examined (Kalinowski 2002), and in this case, this was 0.36. Assigning a value of 0.10 to each stream section would, therefore, dramatically overestimate the observed genetic distance between the first and last population (which averaged 0.36, as expected). The best that the stream tree algorithm could do was to assign each section of the river a genetic distance of approximately 0.06. This underestimated the genetic distance between adjacent populations but did not drastically overestimate the genetic distance between distant populations.

This inability of the stream tree algorithm to accurately depict genetic relationships among highly differentiated populations should not be viewed as a critical shortcoming of the algorithm. The source of the problem is that F_{ST} asymptotically approaches a maximum value as populations become highly diverged. Switching to a genetic distance that has a range of 0 to ∞ may alleviate this problem if genetic differentiation is not too extreme. For the case of the stepping stone model with a migration rate of 0.001, switching to Nei's (1978) distance raised the average R^2 to over 0.99. If, however, genetic differentiation is so extreme that populations have diverged to the point that they share no alleles, this will not solve the problem. The important lesson here is that stream trees (and any summary of population structure) should be interpreted with caution when genetic distances approach their maximum value.

The semi-realistic model of gene flow yielded no surprises. In all of the simulated data sets, the genetic distance assigned to the stream section having the waterfall was the highest value in the stream tree (average = 0.33). The tributary sections had an average genetic distance of 0.12, which was appropriate because they had the second lowest rate of gene flow between them. The stream sections having a migration rate of 0.1 had an average genetic distance of 0.0038, and the main-stem sections had an average of 0.0031. This shows that there is some bias in the main-stem estimates (the correct estimate would be 0.0). However, such bias is inevitable because we constrained genetic distances in stream trees to be nonnegative (the median estimate of the genetic distance for main-stem sections was 0.0, as we would wish). The similarity of the estimates for the main-stem sections and the stream sections (0.0031 vs. 0.0038) shows that there is a limit to how accurately subtle amounts of genetic differentiation can be detected. This is not surprising; from a genetic perspective, a migration rate of 0.1 is very high. In these simulations, this corresponded to 100 migrants per generation. Detecting the subtle amount of genetic differentiation that would persist in the presence of such gene flow, and differentiating it from the case of no differentiation, is possible but requires substantially more than 12 loci (results not shown).

Empirical examples

In three out of four empirical tests that we performed, our stream tree model of genetic differentiation had an R^2 value greater than 0.97 (Table 1). This indicates that the genetic differentiation in three of the four species can be successfully mapped to the stream sections connecting the populations. In one metapopulation of bull trout (Whiteley et al. 2006), the stream tree model of population structure had a modest R^2 of 0.68. We do not have a satisfactory explanation for why the stream tree model was not successful for this metapopulation, but Whiteley et al. (2006) did find an unexpected genetic discontinuity in one of the drainages that they could not explain and suggested that there may have been headwater exchange in another drainage.

The stream tree constructed for rainbow trout in Klickitat watershed was consistent with the geography of the basin (Fig. 6). The largest genetic distances on the stream tree correspond to stream section having waterfalls or beaver dams that are thought to prevent upstream fish passage. The smallest genetic distances on the map generally corresponded to sections of the main-stem Klickitat and to stream sections connecting anadromous populations. Note in particular that each section of the lower main-stem Klickitat River was assigned a genetic distance of zero. This suggests that these stream sections are not even a subtle barrier to gene flow among steelhead.

The four empirical examples showed that stream trees usually did as good of a job of summarizing a matrix of genetic distances as neighbor-joining trees (Table 1). Neighbor-joining trees had higher R^2 values for two out of the four cases. The neighbor-joining tree had a poor fit to the data of Whiteley et al. (2006), as did the stream tree. Other than this, no obvious trends were observed.

Software

We have written a computer program, *StreamTree*, to perform the calculations described in this paper. *StreamTree* runs on the Windows operating system and is available for free download at www.montana.edu/kalinowski. Instructions and a sample input file are available at that Web site.

Discussion

We have presented a novel statistical method to map genetic differences among populations to stream sections of a watershed. The method is conceptually and computationally straightforward. It worked well with simulated data and with three out of the four empirical data sets that we analyzed. In the one empirical example where our model did not provide a good fit for the data, neither did a neighbor-joining tree.

The goal of landscape genetics is to understand how geography affects genetic diversity. Such research often has two steps: the first descriptive and the second explanatory. Stream trees are primarily useful for the first step, describing the spatial distribution of genetic diversity. The stream tree algorithm assigns genetic distances to stream sections

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but does not try to explain how these differences evolved, or even to correlate them with physical characteristics of the river. The only geographic information used by the algorithm is the topology of the watershed. Because of this, stream trees will be equally useful for mapping genetic diversity to stream sections whether genetic diversity has been shaped by geography or by historical processes unrelated to geography.

The stream tree model of population structure could be modified to include geographical features other than stream sections. For example, if two watersheds were separated by a mountain range, the mountain range could be included as a geographic feature "connecting" the populations on each side of the range (provided that this was done in such a way that only one path connected each pair of populations).

The stream tree model assumes that genetic differences between populations accumulate in an additive manner through a watershed. This is not the same as assuming an isolation-by-distance relationship; the stream tree algorithm can assign short stream sections a large genetic distance and long stream section a small genetic distance. The algorithm, however, does assume that genetic differentiation does not decrease with distance. This means that if populations A, B, and C are arranged in a watershed and that population B is between populations A and C, the stream tree algorithm assumes the genetic distance between A and B, D_{AB}, to be less than or equal to the genetic distance between A and C, D_{AC} (the model also assumes that $D_{BC} \leq D_{AC}$). If this assumption is not true, the stream model will not fit the genetic data well.

There are at least a few evolutionary processes that can violate this assumption. First, as shown in the "headwater exchange" simulation, if gene flow between populations A and C occurs outside of the recognized water channels (i.e., headwater exchange or transplant of fish by fisheries managers), populations A and C may be more similar to each other than to population B. Alternatively, the three populations may have historically been connected by high rates of gene flow, but then became isolated from each other. If population B experiences a population bottleneck that causes rapid genetic drift, and populations A and C retain a large effective population size, populations A and C might be the most genetically similar. Lastly, populations A and C might have a different life history than population B and may have more gene flow between them than to population B. Other evolutionary scenarios that create patterns are possible, but populations having such distributions of genetic diversity are probably less common than populations having spatially additive relationships.

As we have noted above, the stream tree algorithm only provides a map of genetic differences among populations. We anticipate that more geographically explicit models will be constructed in which the genetic distance between populations is explicitly related to stream width, gradient, or other physical variables. This will be more helpful in identifying how landscape characteristics shape genetic variation among populations, which, of course, is the ultimate goal of landscape genetics.

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Appendix A

Table A1. An example data file that could be used to construct a stream tree using linear regression for the populations depicted in Fig. 1.

COMP	DOBS	SEC_1	SEC_2	SEC_3	SEC_4	SEC_5	SEC_6
AB	0.17	1	1	1	0	0	0
AC	0.07	1	1	0	0	0	0
AD	0.13	1	0	0	1	1	0
AE	0.07	1	0	0	1	0	1
BC	0.10	0	0	1	0	0	0
BD	0.22	0	1	1	1	1	0
BE	0.16	0	1	1	1	0	1
CD	0.12	0	1	0	1	1	0
CE	0.06	0	1	0	1	0	1
DE	0.08	0	0	0	0	1	1

Note: The column at far left, COMP, lists the populations being compared. This is useful to keep the data organized but is not used in the regression. The next column, DOBS, is the observed genetic distance for each pair of populations. The columns SEC_1 to SEC_6 contain 0s and 1s that indicate whether each section of stream (1 to 6) is between the populations listed in the COMP column. When conducting the regression, DOBS is the dependent variable and SEC_1 through SEC_6 are the independent variables. There is no intercept. The output from the regression will be a set of six coefficients (0.04, 0.03, 0.10, 0.02, 0.05, 0.01). These are the genetic distances to assign to each section of stream in the watershed.

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Pond hydroperiod alters the effect of density-dependent processes on larval anurans

Tracy Noel Rogers and David R. Chalcraft

Abstract: Many studies examine how changes (disturbances) to the abiotic environment alter the intensity of species interactions by directly changing the numbers of individuals involved in the interaction but few studies examine how the abiotic environment affects how strongly individuals interact with each other. We conducted an experiment to evaluate how pond hydroperiod affected the ability of two larval anuran species (*Hyla squirella* and *Bufo terrestris*) to interact with intraspecific and interspecific competitors. Pond hydroperiod altered how an increase in the abundance of intraspecific competitors affected fitness components of *B. terrestris* but not for *H. squirella*. Specifically, an increase in intraspecific abundance caused a reduction in *B. terrestris* survivorship in short hydroperiod ponds but had no effect on fitness components of *B. terrestris* in long hydroperiod ponds. Interspecific competition did not occur between these species in either short or long hydroperiod ponds. Our study highlights the point that the strength of density-dependent processes operating in the aquatic environment can depend on pond hydroperiod. Consequently, the extent to which processes operating in the aquatic environment contribute to the regulation of population and metapopulation size for amphibians will also likely depend on pond hydroperiod.

Résumé : De nombreuses études examinent comment les changements (perturbations) dans l'environnement abiotique modifient l'intensité des interactions entre les espèces en changeant directement le nombre d'individus impliqués dans l'interaction, mais peu de travaux examinent comment l'environnement abiotique affecte la force de l'interaction entre les individus. Nous avons monté une expérience pour évaluer comment l'hydropériode d'un étang affecte la capacité des larves de deux espèces d'anoures (*Hyla squirella* et *Bufo terrestris*) d'interagir avec leurs compétiteurs intraspécifiques et interspécifiques. L'hydropériode de l'étang change la manière par laquelle une augmentation de nombre de compétiteurs intraspécifiques affecte les composantes de la fitnesss chez *B. terrestris*, mais non chez *H. aquirella*. De façon plus spécifique, une augmentation de l'abondance au sein de l'espèce cause une réduction de la survie de *B. terrestris* dans les étangs à courte hydropériode, mais reste sans effet sur les composantes de la fitness de *B. terrestris* dans les étangs à longue hydropériode. Il ne se produit pas de compétition interspécifique entre ces espèces dans les étangs, que l'hydropériode soit courte ou longue. Notre étude souligne le fait que la force des processus dépendants de la densité en opération dans le milieu aquatique peut dépendre de l'hydropériode de l'étang. En conséquence, l'importance de la contribution des processus en œuvre dans le milieu aquatique à la régulation de la taille de la population et de la métapopulation chez les amphibiens dépendra aussi vraisemblablement de l'hydropériode de l'étang.

[Traduit par la Rédaction]

Introduction

Extensive research demonstrates that both the abiotic environment and biotic interactions have important direct effects on the distribution and abundance of species (for reviews, see Schoener (1983), Sih et al. (1985), and Gurevitch et al. (1992)). Less appreciated, however, are ways that the abiotic environment can alter how biotic interactions affect a species (Dunson and Travis 1991; Chalcraft and Andrews 1999). The abiotic environment can alter biotic interactions in two ways (Wootton 1994; Chalcraft and Andrews 1999). First, the abiotic environment can alter the intensity of either interspecific or intraspecific interactions by changing the abundances of species involved in the interaction. Second, the abiotic environment can change the per capita effect that either intraspecific or interspecific individuals have on a species (i.e., an environmentally contingent interaction). Although ecologists have devoted much attention to the first mechanism (for reviews on the intermediate disturbance hypothesis, see, e.g., Sousa (1984) and Chesson (2000)), less attention has been directed toward environmentally contingent interactions. Here we examine how an abiotic factor, pond hydroperiod, affects intraspecific and interspecific competitive interactions involving two species of larval anurans.

Hydroperiod and competition have important direct effects on larval anurans. The most obvious effect of hydroperiod is death if larvae do not metamorphose into terrestrial adults before the pond dries. Both hydroperiod and competition also have important effects on growth and

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development of larval anurans. For example, larval anurans tend to metamorphose at smaller sizes in ponds with a shorter hydroperiod (Wilbur 1987; Crump 1989; Laurila and Kujasalo 1999) and in ponds with more competitors (Wilbur 1977, 1982, 1987). Metamorphosing at a smaller mass can decrease adult fitness by lowering survival and fecundity and delaying the onset of sexual maturation (Banks and Beebee 1986; Smith 1987; Berven 1990).

Hydroperiod can also affect the intensity of biotic interactions imposed on larval anurans by altering abundances of predator or competitor species (for review, see Wellborn et al. 1996) or by causing the effects of competitive interactions to be more severe. For example, Wilbur (1987) found that an increase in competitor abundance could slow larval development sufficiently so that no larvae could complete metamorphosis in ponds that dry quickly, while no larvae would die as the result of desiccation in permanent ponds despite reduced developmental rates. Pond drying may intensify competitive interactions by reducing phytoplankton and periphyton abundance (by reducing the volume of water present or the amount of substrate surface area that is underwater), the two major food sources for larval anurans (Alford 1999), while increasing the encounter rate between potential competitors.

Although Wilbur (1987) clearly demonstrated that shortening pond hydroperiod could amplify the intensity of competitive interactions, his study did not separate the effects of interspecific or intraspecific competitors. Tejedo and Reques' (1994) study on the natterjack toad demonstrated that the strength of intraspecific density dependence was stronger in short hydroperiod ponds, but their study only focused on one species and did not examine the influence of hydroperiod on interspecific competition. In our study, we examined how hydroperiod affected the intensity of intraspecific and interspecific interactions involving two species of larval anurans. We hypothesized that pond drying will intensify the effect of intraspecific and interspecific interactions on larval anurans.

Study species

Our study focused on the effects of hydroperiod and intraspecific and interspecific interactions on larvae of *Hyla squirella* (squirrel tree frog) and *Bufo terrestris* (southern toad). Both *H. squirella* and *B. terrestris* are summer breeders that oviposit in ponds with relatively short hydroperiods, but *B. terrestris* will also oviposit in ponds with longer hydroperiods (for review, see Lannoo 2005). Furthermore, both species graze on algae and detritus that are either suspended in the water column or occur on substrates and have comparable larval developmental times (for review, see Lannoo 2005). Given the extensive similarities, it is likely that *H. squirella* and *B. terrestris* are competitors. Although both species are common in the coastal plain of the southeastern USA, little is known regarding how they interact with each other and how these interactions may vary in ponds that differ in hydroperiod.

Materials and methods

Experimental design

We conducted an experiment in 48 artificial ponds (modified 1100 L Rubbermaid cattle-watering tanks) to evaluate the effect of hydroperiod on the relative strengths of intraspecific and interspecific interactions between *H. squirella* and *B. terrestris*. Control over the experimental conditions in independent and replicate artificial ponds allows investigators to explore complex ecological questions that would otherwise be impractical to study in natural ponds (Chalcraft et al. 2005). Numerous studies suggest that processes identified to be important to the larval ecology of amphibians in artificial ponds similar to ours are also important in natural ponds (Scott 1990; Semlitsch et al. 1996; Resetarits and Fauth 1998).

Our experimental design involved manipulations of the initial abundance of both species in ponds that dried in 50 days (short hydroperiod) and in ponds that did not dry (long hydroperiod). The duration of the short hydroperiod ponds should have allowed most of the tadpoles to metamorphose but under stressful drying conditions, whereas individuals in long hydroperiod ponds did not experience drying conditions. Within each hydroperiod, we established six treatments that differed in the initial abundances of each species: (*i*) no larval anurans of either species, (*ii*) 200 *H. squirella* alone, (*iii*) 400 *H. squirella* alone, (*iv*) 200 *B. terrestris* alone, (*v*) 400 *B. terrestris* alone, and (*vi*) 200 *B. terrestris* and 200 *H. squirella*. This experimental design allowed us to measure (*i*) the effect of intraspecific interactions and (*ii*) the relative effect of intraspecific and interspecific interactions. Treatments without larval anurans allowed us to quantify the availability of algal resources in the absence of grazing larval anurans. The initial densities used in this experiment fell within the range of those observed in natural ponds (Morin 1983; Travis and Trexler 1986; Wilbur 1987). Each of the 12 treatments was replicated four times in a randomized complete block design.

Field techniques

Four groups of 12 closely spaced artificial ponds were placed in an open field at East Carolina University's West Research Campus in Greenville, North Carolina, and each group was identified as a different statistical block. All procedures (e.g., creation, experimental manipulation, and monitoring of ponds) were conducted on a block-by-block basis to minimize within-block variation not attributable to treatment differences. Each artificial pond was equipped with an L-shaped polyvinyl chloride (PVC) standpipe to allow for controlled draining of the pond. The standpipe opening was covered with a screen to prevent unwanted colonization and escape of experimental animals. Ponds were filled with water to a depth of 35 cm on 13–14 May 2006. To add further realism to these artificial ponds, we added 500 g of hardwood leaf litter and an inoculation of algae and zooplankton obtained from a natural pond. Each pond was covered with a tight-fitting fiberglass screen lid to prevent the colonization of unwanted animals and escape of experimental animals.

Larval anurans were obtained from 15 clutches of *H. squirella* and four clutches of *B. terrestris* that were laid 3–6 June 2006. Before the addition of larval anurans to artificial ponds, we randomly assigned a different treatment designation to each artificial pond within each block. Three-day-old larval anurans were randomly assigned to each arti-

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ficial pond with the constraint that (*i*) each pond receive a similar proportion of individuals from each clutch to ensure similar levels of genetic diversity within each pond and (*ii*) the number of individuals of each species added to a pond corresponds to the treatment assigned to that pond.

Although all ponds initially had the same depth (35 cm), we simulated drying in short hydroperiod ponds by lowering the L-shaped PVC pipe installed in each pond at predetermined intervals. The rate of drying was based on an equation describing the natural drying curve of ponds located in North Carolina (Wilbur 1987):

$$D_j = 1 - (j/t)^a P$$

where D_j is the desired depth on day j, j is the time in days since the larval anurans were added to ponds, t is the target date in days for the depth to reach zero, P is the depth at the beginning of the experiment, and a is a shape parameter ($a = 2$) for the drying curve.

We quantified algal concentrations present in each pond 40 days after larval anurans were added. A periphyton sample was obtained by scraping algae off a piece of flagging tape tied to a ceramic tile at the bottom of each pond. We recorded the amount of surface area scraped for each sample. A phytoplankton sample was obtained by filtering a known volume of water from each pond. Periphyton and phytoplankton samples were frozen for later chlorophyll extraction using the spectrophotometric methods for chlorophyll *a* described by Clesceri et al. (1998).

Ponds were checked daily, and newly metamorphosed frogs or toads (metamorphs), defined as individuals with at least one forelimb, were removed and returned to the lab. After tail resorption, each metamorph was blotted dry with a damp cloth, measured to the nearest 0.001 g, and then released at the site of egg collection. Larval period of each metamorph was estimated from day of hatching until the date at which it was removed from the pond. Short hydroperiod ponds were terminated on day 50 at a depth of 5 cm as the remaining tadpoles would not likely survive to metamorphose before complete pond drying. Long hydroperiod ponds were terminated on 23 September 2006 (day 104) after all metamorphs emerged. Survivorship for each species was measured as the proportion of individuals added to a pond that successfully metamorphosed. Some larvae survived to the end of the experiment in short hydroperiod ponds but did not complete metamorphosis.

Statistical analyses

Each pond represented an independent experimental unit from which mean larval period, mean mass at metamorphosis, and survivorship to metamorphosis of each species was measured and used for statistical analyses. Survivorship data were log-transformed as this provided a measure of instantaneous per capita mortality rates and homogenized variances. No *B. terrestris* successfully metamorphosed in one replicate of the treatment with a high abundance of *B. terrestris* in a short hydroperiod pond. As a result, we added a nominal value (0.01) to all *B. terrestris* survival data before transformation so that this observation could be included in the analysis. Excluding this particular treatment from the analysis did not qualitatively change the results. Mass at metamorphosis was log-transformed to meet the assumption of homogeneity of variance. There were no problems with normality of residuals or homogeneity of variances for untransformed values of larval period.

We analyzed our data with analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA). Experimental blocks were treated as random effects in ANOVA and MANOVA models. All other terms in ANOVA and MANOVA models were treated as fixed effects. All analyses were conducted in SYSTAT (version 11.0; Systat Software Inc., Chicago, Illinois). We consider effects to be significant when $p < 0.05$, but we also discuss trends in effects when $p < 0.10$. Summary p values are provided in the manuscript, but the full MANOVA results are available on the Web as supplemental tables.[2]

We tested the hypothesis that hydroperiod alters the effect of increasing intraspecific abundance on a response vector describing larval growth components (larval period and mass at metamorphosis) by conducting a factorial MANOVA for each species with intraspecific abundance (200 vs. 400 larval anurans), hydroperiod (short vs. long), and block specified as the main factors (Smith 1987; Fauth and Resetarits 1991; Chalcraft and Resetarits 2003). These MANOVAs only included data from treatments that did not contain an interspecific competitor, and a significant interaction between intraspecific abundance and hydroperiod supports the hypothesis that hydroperiod alters the effect of increasing intraspecific abundance. Following MANOVA, we used factorial ANOVA, with the same statistical model, to determine if either larval period, mass at metamorphosis, or log survivorship to metamorphosis was affected by these intraspecific treatments.

Similarly, we tested the hypothesis that hydroperiod alters the effects of interspecific interactions on the response vector describing components of larval growth (larval period and mass at metamorphosis) by conducting a factorial MANOVA for each species, with the presence of an interspecific competitor, hydroperiod, and block specified as the main factors. The effect of an interspecific competitor was tested by comparing growth components and survival of a species when it occurred by itself at an initial abundance of 200 individuals versus when that species occurred with a potential interspecific competitor (i.e., treatments with 200 larvae of each species). A significant interaction between the presence of an interspecific competitor and hydroperiod supports the hypothesis that the effect of interspecific interactions changes with hydroperiod. Following MANOVA, we used factorial ANOVA, with the same statistical model, to determine if larval period, mass at metamorphosis, or the log survivorship to metamorphosis was affected by interspecific competitors.

Two additional ANOVAs (one for periphyton and one for phytoplankton) were conducted to evaluate the effects of larval anuran presence, hydroperiod, and their interaction on

[2] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 3857. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/cms/unpub_e.html.

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the availability of algal resources. The total degrees of freedom for these analyses were reduced because of the loss of three phytoplankton samples and two periphyton samples. When the presence of larval anurans altered algal resources, we performed two additional ANOVAs. One factorial ANOVA evaluated the effects of varying species composition (i.e., *H. squirella* alone vs. *B. terrestris* alone vs. both together) in treatments with 400 larval anurans and the interactive effect between species composition and hydroperiod. The other factorial ANOVA only used data with a single species present and evaluated the effects of species identity (*H. squirella* vs. *B. terrestris*), abundance (200 vs. 400), hydroperiod, and their two-way interactions.

Results

There was a trend for hydroperiod to affect growth components of *B. terrestris* when it occurred by itself, but univariate tests did not detect a significant effect of hydroperiod on either larval period or mass at metamorphosis ($p \geq 0.465$; Supplemental Table S1[2]; Figs. 1*a*, 1*b*). Furthermore, growth components of *B. terrestris* were not affected by either intraspecific density or the interaction between intraspecific density and hydroperiod when *B. terrestris* occurred by itself ($p \geq 0.294$; Supplemental Table S1[2]; Figs. 1*a*, 1*b*). The influence of intraspecific density on *B. terrestris* survival depended on hydroperiod ($p = 0.009$; Supplemental Table S1[2]; Fig. 1*c*). Specifically, an increase in *B. terrestris* density reduced survivorship in short hydroperiod ponds ($F_{[1,3]} = 53.356$, $p = 0.005$) but not in long hydroperiod ponds ($F_{[1,3]} = 1.561$, $p = 0.300$). Neither the presence of *H. squirella* nor the interaction between *H. squirella* presence and hydroperiod affected growth components or survival of *B. terrestris* ($p > 0.184$; Supplemental Table S2[2]; Fig. 1).

Although a MANOVA suggested that neither intraspecific density nor hydroperiod nor their interaction affected growth components of *H. squirella* when it occurred by itself, univariate ANOVA indicated that *H. squirella* experienced a decrease in mean mass as conspecific density increased ($p = 0.021$; Supplemental Table S3[2]; Figs. 2*a*, 2*b*). Larval period and survival of *H. squirrella* did not differ significantly among treatments that contained only *H. squirrella* ($p \geq 0.152$; Supplemental Table S3[2]; Figs. 2*b*, 2*c*). Neither the presence of *B. terrestris* nor the interaction between *B. terrestris* presence and hydroperiod affected growth components or survival of *H. squirella* ($p \geq 0.205$; Supplemental Table S4[2]; Fig. 2).

Larval anurans reduced periphyton concentrations ($p = 0.041$; Supplemental Table S5*a*[2]; Fig. 3*a*). Although there was a trend for the concentration of periphyton to be greater in long hydroperiod ponds ($p = 0.074$), the interactive effect between larval anuran presence and hydroperiod was not significant ($p > 0.704$; Supplemental Table S5*a*[2]; Fig. 3*a*). When 400 larval anurans were present, neither species composition nor hydroperiod nor their interaction affected periphyton concentration ($p \geq 0.168$; Supplemental Table S5*b*[2]). Neither hydroperiod nor intraspecific density nor species identity nor any of their two-way interactions affected periphyton concentration in treatments that only contained a single larval anuran species ($p \geq 0.187$; Supplemental Table S5*c*[2]). Phytoplankton concentration was not altered by the presence of larval anurans, hydroperiod, or their interaction ($p \geq 0.292$; Supplemental Table S5*d*[2]; Fig. 3*b*).

Fig. 1. (*a*) Larval period, (*b*) mass at metamorphosis, and (*c*) survivorship of *Bufo terrestris* larvae in ponds varying in hydroperiod and the density of potential competitors (open bars, 200 *B. terrestris*; shaded bars, 400 *B. terrestris*; solid bars, 200 *B. terrestris* and 200 *Hyla squirella*). Each bar (±1 standard error) represents the mean of four replicates of a particular treatment.

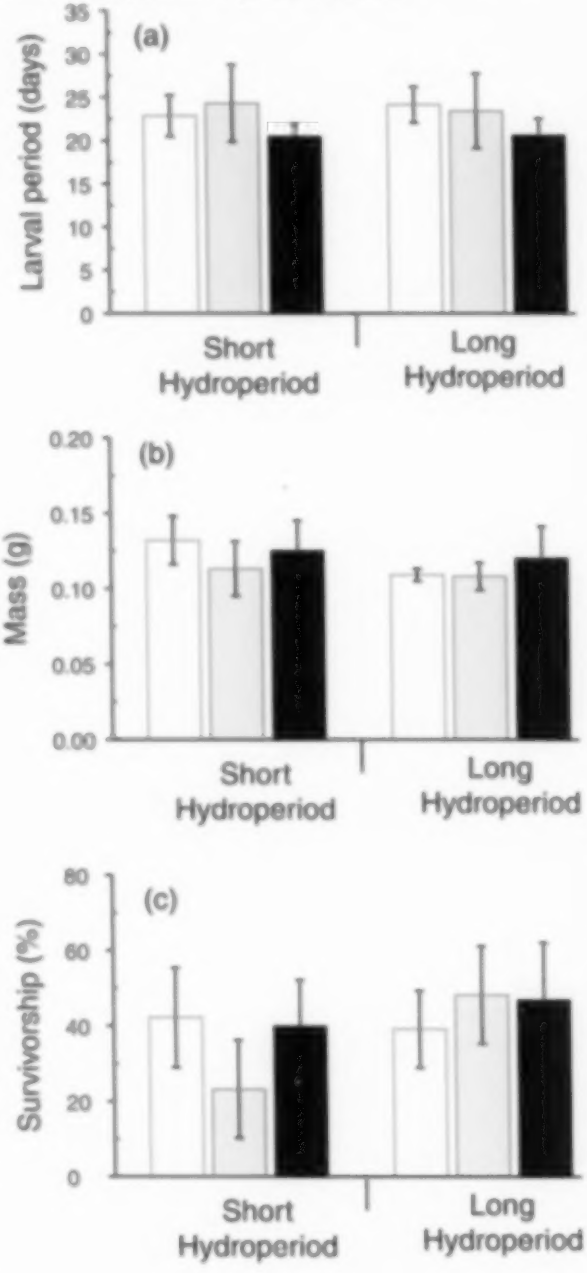

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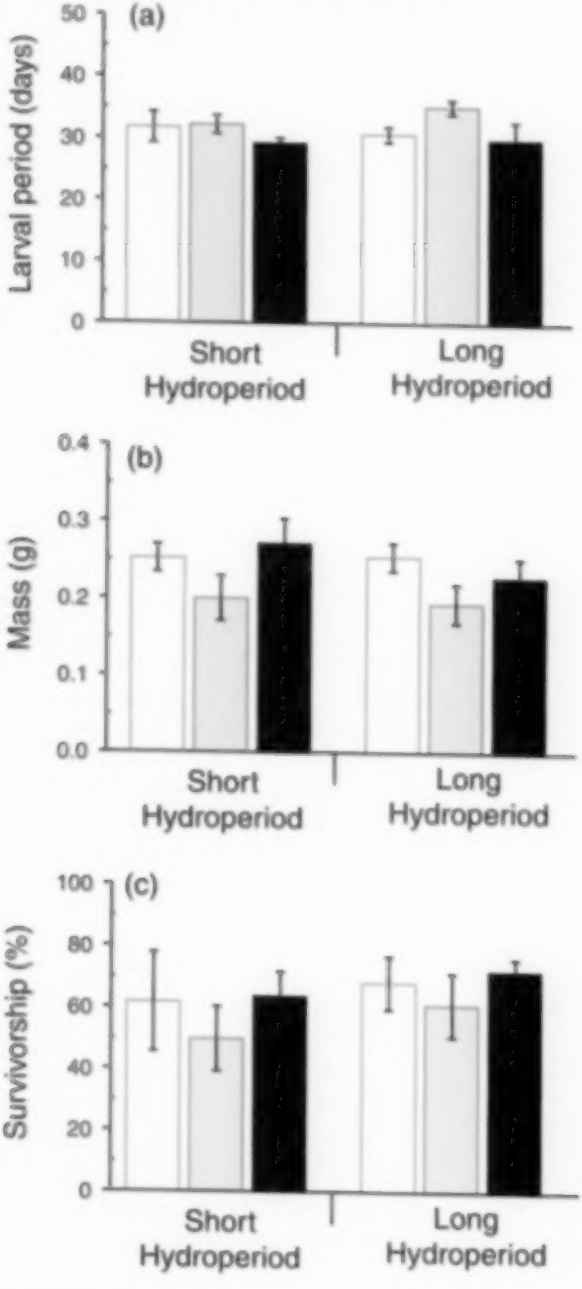
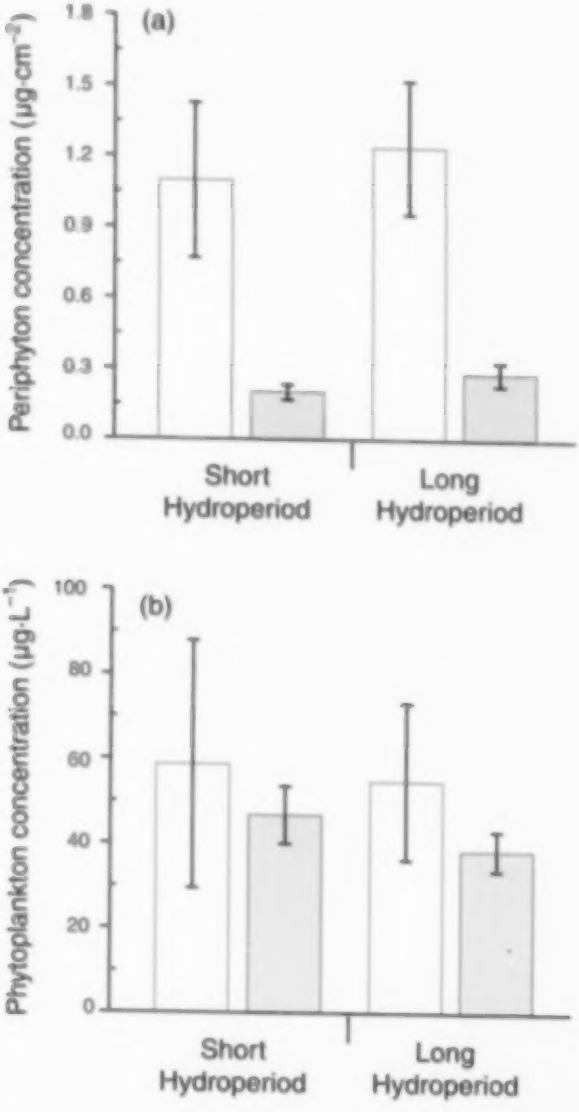
Fig. 2. (*a*) Larval period, (*b*) mass at metamorphosis, and (*c*) survivorship of *Hyla squirella* larvae in ponds varying in hydroperiod and the density of potential competitors (open bars, 200 *H. squirella*; shaded bars, 400 *H. squirella*; solid bars, 200 *H. squirella* and 200 *Bufo terrestris*). Each bar (±1 standard error) represents the mean of four replicates of a particular treatment.



Discussion

Density-dependent changes in per capita birth, death, or migration rates are essential in maintaining population sizes about some equilibrium value(s). We detected such density-dependent changes during the larval stage of two anuran species. Increasing *H. squirella* abundance reduced *H. squirella* mass at metamorphosis, and prior studies (Banks and Beebee 1986; Smith 1987; Berven 1990) have demonstrated that reduced mass at metamorphosis correlates to reduced fecundity (i.e., per capita birth rates) in many species of larval anurans. Furthermore, increasing the abundance of *B. terrestris* in short hydroperiod ponds resulted in a reduc-

Fig. 3. Mean concentration of (*a*) periphyton (±1 standard error) and (*b*) phytoplankton (±1 standard error) collected from short and long hydroperiod ponds that had larval anurans either absent (open bars) or present (shaded bars): $n = 4$ in ponds with no larval anurans; $n = 18$ for periphyton in ponds with larval anurans; $n = 19$ for phytoplankton in long hydroperiod ponds with larval anurans; and $n = 16$ for phytoplankton in short hydroperiod ponds with larval anurans.



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tion in the proportion of *B. terrestris* that successfully metamorphosed from a pond (i.e., per capita death rates). Consequently, variation in the number of larval anurans present alters per capita birth (*H. squirella*) and death rates (*B. terrestris*), which could regulate the population size of these species. Such density dependence during the larval stage has been documented for many anuran species (for reviews, see Alford (1999) and Skelly and Kiesecker (2001)).

Most interestingly, we also found that the strength of intraspecific density dependence depended on the abiotic environment. Specifically, the effect of increasing intraspecific abundance on *B. terrestris* depended on whether intraspecific interactions occurred within a short hydroperiod or a long hydroperiod pond. An increase in the initial abundance of *B. terrestris* caused a decline in their survivorship in short hydroperiod ponds but had no (or very weak) effect on any of their fitness components in long hydroperiod ponds. Although a number of studies are now documenting the extent of spatial and temporal variation in the strength of density-dependent processes (e.g., Bjornstad et al. 1995; Agrawal et al. 2004; Underwood 2007), the source of this variation remains unclear (Agrawal et al. 2004). Our results have identified that a source of variation in the effect of intraspecific density dependence was an abiotic component of the habitat (i.e., hydroperiod).

The effect of the abiotic environment on the strength of intraspecific density dependence is important for at least two reasons. First, it demonstrates that results from studies on biotic processes in one habitat may not necessarily translate to other habitats. During the past 30 years, ecologists have become increasingly aware that the biotic context in which a particular species interaction is measured can actually change the intensity of the species interaction (for reviews, see Wootton (1994) and Werner and Peacor (2003)). Although the dependence of species interactions on the abiotic context was introduced some time ago (Tansley 1917; Park 1954), the importance of such environmentally contingent interactions has not received much consideration. By understanding how the abiotic context affects the intensity of species interactions, spatial and temporal variation in the intensity of biotic interactions become predictable rather than idiosyncratic.

Second, geographic variation in the strength of density-dependent processes that operate during a particular life history stage could change the relative importance of different life history stages to population regulation. The presence of density-dependent interactions among larvae of *B. terrestris* within short but not long hydroperiod ponds suggests that the influence of larval (aquatic) ecology on regulating the size of *B. terrestris* populations will depend on pond hydroperiod. Specifically, larval *B. terrestris* may regulate population size in short hydroperiod ponds, but processes operating on terrestrial phases of *B. terrestris* may be more important in regulating population size at long hydroperiod ponds. Furthermore, the influence of *B. terrestris* larvae on the regulation of metapopulation size may be dependent on the frequency distribution of hydroperiod length among ponds occurring within a metapopulation. If most ponds in a particular metapopulation have a long hydroperiod, the aquatic environment may play only a minor role in regulating the size of the *B. terrestris* metapopulation. Instead, those metapopulations would be influenced more by density dependence occurring during the adult terrestrial stage. Conversely, if most ponds in a particular metapopulation have a short hydroperiod, larval *B. terrestris* may play a much more important role. Alternatively, processes other than intraspecific competition (e.g., predation) may be responsible for regulating larval population sizes of *B. terrestris* in ponds with long hydroperiods.

Why does hydroperiod affect intraspecific interactions among larval *B. terrestris*? We suggest that the increase in the abundance of *B. terrestris* in short hydroperiod ponds causes the total amount of algal food resources available per individual to drop below a threshold value so that proportionately fewer larvae could sustain themselves. This threshold did not appear to be crossed in the long hydroperiod ponds, as survivorship was similar in long hydroperiod ponds that differed in the initial abundance of larval anurans. We have two pieces of evidence for the idea that the total amount of algal food resources available per individual is reduced in short hydroperiod ponds. First, we found that the concentration of periphyton tended to be lower in short hydroperiod ponds. Second, and more importantly, as short hydroperiod ponds dried, there was less submerged surface area available for periphyton growth. Reduced submerged surface area occurred because drying exposed more of the sides of the tanks to the air (5 and 35 cm of a tank side were submerged at the end of the experiment in short and long hydroperiod ponds, respectively). Reductions in submerged surface area also occur as natural ponds dry. Consequently, short hydroperiod ponds had lower total amounts of periphyton present. Thus, an increase in the initial abundance of *B. terrestris* in short hydroperiod ponds means that the amount of food resources per individual is lower in ponds with more larval anurans. An increase in the initial abundance of *B. terrestris* in long hydroperiod ponds does reduce the amount of algal resources per individual, but given the greater abundance of algal resources in long hydroperiod ponds, each individual larva in long hydroperiod ponds would have more food available to it than an individual larva in short hydroperiod ponds. It must be noted, however, that our metric of standing algal biomass may not be an accurate reflection of productivity (i.e., instantaneous rates of biomass production) and other hypotheses could also explain these data.

Hydroperiod altered the effects of intraspecific interactions among *B. terrestris* but not among *H. squirella*. Consequently, hydroperiod does not consistently alter the strength of intraspecific interactions for different anuran species. Our work, in conjunction with the work of Tejedo and Reques (1994), however, suggests that a significant effect of hydroperiod on the strength of intraspecific interactions is not uncommon among anuran species. Further work is necessary to identify properties of species that make their fitness components more susceptible to changes in population abundance in short hydroperiod ponds. One trait that could explain differences in how hydroperiod affects the strength of intraspecific interactions is foraging location. Benthic larval anurans would not really experience a change in the total amount of food resources available in a drying pond because algae that was present higher up in the water column was never really available to them so they are not

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losing resources as water volume declines. Microhabitat generalists would experience a decline in resource availability as ponds dry, however, because there is reduced water volume in which algae can grow.

Prior studies of larval anurans in the southeastern US have often documented that interspecific interactions among larval anurans can be relatively intense (for reviews, see Alford (1999) and Skelly and Kiesecker (2001)). The interspecific interactions that we observed between *H. squirella* and *B. terrestris* were weak. Consequently, interspecific interactions during the larval stage likely will not play an important role in determining whether *B. terrestris* and *H. squirella* co-occur. Although other studies suggest that larvae of various *Bufo* species are relatively insensitive to the effects of interspecific interactions (Licht 1967; Wilbur 1977; Richter-Boix et al. 2004), the absence of an interspecific effect of *B. terrestris* on *H. squirella* was surprising; prior studies (e.g., Morin 1983; Wilbur 1987; Fauth and Resetarits 1991) suggest that larval *Bufo* interact strongly with other hylid species. Richardson (2001), however, measured 14 different larval traits in five hylid species from the southeastern US and found that *H. squirella* differed substantially in these traits from the other hylid species that she studied (*H. cinerea*, *H. femoralis*, *H. gratiosa*, and *H. versicolor*). Consequently, these differences in phenotypic traits may explain why *H. squirella* and *B. terrestris* did not interact in similar ways as other hylids do with *B. terrestris*.

Although hydroperiod affected how an increase in intraspecific abundance affected the survival of *B. terrestris*, hydroperiod did not appear to affect the larval growth of either species. This result was rather surprising as both species can often be found in ponds that dry relatively rapidly, and hydroperiod has been found to affect growth in other species that use temporary habitats (e.g., Blaustein et al. 1999). This suggests that these species either lack the ability to detect pond drying or that our "short" hydroperiod length was not sufficiently short to elicit a growth response. Our choice for short hydroperiod duration was based in part, however, on the midpoint of the described larval period for *H. squirella* (for review, see Lannoo 2005) and the fact that this duration was used for other larval anuran studies conducted in the southeastern US (e.g., Wilbur 1987).

In summary, we found that an increase in intraspecific abundance has an important effect on larval performance of two anuran species but that the effect of increasing intraspecific abundance on larval performance can depend on the abiotic environment. The main implications of our study are (*i*) that geographic variation in abiotic conditions can alter the relative importance of particular life history stages to population regulation and (*ii*) that studies of species interactions (e.g., intraspecific competition) conducted under one set of abiotic conditions may not accurately predict the effect of species interactions under a different set of environmental conditions. Our results indicate that a better understanding of how intraspecific and interspecific interactions affect organisms will require more attention to the various mechanisms through which abiotic factors affect ecological communities.

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Acoustic characterization of *Mysis relicta* at multiple frequencies

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Abstract: We measured acoustic backscattering from *Mysis relicta*, a common invertebrate in northern lakes, using five frequencies (38, 120, 200, 430, and 710 kHz). Acoustic backscattering from mysids was highest at 430 kHz and lowest at 38 kHz (19 dB lower). Maximum difference between the four other frequencies was 5.2 dB. Mysid target strength (TS) ranged from –80.1 dB at 430 kHz to –99.4 dB at 38 kHz (12 mm average length, range 5–21 mm). A theoretical scattering model (Stanton's fluid-like, bent-cylinder model) predicted TS within 0.3–1.9 dB of observed TS for the different frequencies. The detection range was lowest at 38 and 710 kHz and greatest at 120 and 200 kHz. Fish were common above the mysid layer and produced higher acoustic backscattering at 38 kHz than at the other frequencies. A combination of 38 kHz and 120 or 200 kHz provides a strong contrast between mysid and fish acoustic backscattering that would help separate these groups using acoustic data.

Résumé : Nous avons mesuré la rétrodiffusion acoustique causée par *Mysis relicta*, un invertébré commun dans les lacs nordiques, à cinq fréquences (38, 120, 200, 430 et 710 kHz). La rétrodiffusion acoustique par les mysidés est maximale à 430 kHz et minimale à 38 kHz (inférieure de 19 dB). La différence maximale entre les quatre autres fréquences est de 5,2 dB. L'indice de cible (TS) des mysidés varie de –80,1 dB à 430 kHz à –99,4 dB à 38 kHz (longueur moyenne 12 mm, étendue 5–21 mm). Un modèle théorique de rétrodiffusion (le modèle du cylindre fluide courbe de Stanton) prédit des valeurs de TS situées entre 0,3 et 1,9 dB des TS observés aux diverses fréquences. La portée de détection est minimale à 38 kHz et 710 kHz et maximale à 120 kHz et 200 kHz. Les poissons sont communs au-dessus de la couche de mysidés et produisent une rétrodiffusion acoustique plus importante à 38 kHz qu'aux autres fréquences. Une combinaison de 38 kHz et de 120 ou 200 kHz donne un fort contraste entre la rétrodiffusion par les mysidés et par les poissons, ce qui aiderait à séparer ces groupes à l'aide de données acoustiques.

[Traduit par la Rédaction]

Introduction

Members of the *Mysis relicta* species flock are up to 25 mm long, benthopelagic crustaceans that are common in cold-water lakes in both North American and Eurasia. They are omnivores that feed on zooplankton and phytoplankton (Grossnickle 1982) and perform diel vertical migrations from their daytime residence on the bottom of lakes to the lower metalimnion at night to feed on zooplankton when predation risk from visually feeding fish is minimized (Levy 1991). Mysids are a major component of many food webs, including large systems such as the Laurentian Great Lakes (Johannsson et al. 2003; Pothoven et al. 2004) and the Baltic Sea (Rudstam et al. 1992*a*), as well as smaller lakes on both continents (Langeland et al. 1991; Chipps and Bennett 2000). The biomass of mysids can be similar to or even exceed the biomass of planktivorous fish (Gal et al. 2006), and the introduction of the species to smaller lakes has led to changes in zooplankton composition and associated food webs (Lasenby et al. 1986; Spencer et al. 1991). Given their importance in lake ecosystems, we need to develop better methods to estimate their abundance.

Although hydroacoustics have been used for quantitative estimates of euphausiids and other marine invertebrates for decades (Greene et al. 1991; Everson et al. 1993; Foote and Stanton 2000), applications to freshwater mysids have been mostly limited to qualitative assessment of vertical migrations (Teraguchi et al. 1975; Levy 1991). However, other similar-sized invertebrates have been estimated quantitatively with acoustics (amphipods: Rudstam et al. 1992*b*; Trevorrow and Tanaka 1997; *Chaoborus* larvae: Eckmann 1998; Kubečka et al. 2000; Malinen et al. 2005; copepods

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Table 1. Configuration of the five frequencies and used in the analysis.

Frequency (kHz)	Type of beam	Half-power beam width (°)	Source level at 1 m (dB re 1 μPa)	Acoustic power (W)	Pulse length (ms)	Absorption (dB·km^{-1}) at 10 °C	Noise level (dB) at 1 m depth (*S*$_v$ domain)
38	Split	7.0×6.9	219.8	100	0.512	0.45	−142
120	Split	7.2×7.3	216.9	150	0.512	4.5	−141
200	Split	6.5×6.5	217.5	90	0.512	12.4	−139
430	Single	6.0×6.0	218.5	51.8	0.500	57.5	−123
710	Single	5.4×5.4	218.2	80	0.512	156.7	−129

Note: For 430 kHz, a BioSonics DtX unit was used; for the other four frequencies, Simrad EY60 units were used. All units operated at 2 pings·s^{-1}. Absorption coefficient was calculated according to Francois and Garrison (1982) at 10 °C. Noise levels were measured using passive recordings.

and cladocerans: Hembre and Megard 2003; Holbrook et al. 2006), and acoustic methods should also be applicable to quantitative estimates of mysid abundance. Estimates of mysid biomass through vertical or horizontal net tows is labor intensive given the large number of samples necessary to measure their typically patchy distributions (*e.g.*, Johannsson et al. 2003). Recently, Gal et al. (2006, with 420 kHz) and Rudstam et al. (2008, with 120 kHz) used acoustics to measure absolute abundance of mysids in Lake Ontario.

Quantitative estimates of abundance with hydroacoustics require knowledge of the target strength (TS, dB re 1 m^2) of the animals insonified. For estimates of invertebrates like mysids that are relatively weak scatterers, we also need to remove the contribution to backscattering from fish. Further, TS is frequency-dependent, and this frequency response has been used for classification of different marine organisms (Cochrane et al. 1991; Korneliussen and Ona 2003), but little information is available on the frequency response of freshwater animals. Fish with swim bladders show a weak frequency response at frequencies typically used in marine (38–200 kHz; Fernandes et al. 2006; Fässler et al. 2007) and freshwater (70–200 kHz; Degan and Wilson 1995; Guillard et al. 2004) applications. In contrast, the phantom midge larva *Chaoborus* has a dramatic frequency response dominated by a strong scattering at 200 kHz (Knudsen et al. 2006). The frequency response of mysids is largely unknown. Information on the TS of mysids over a wide frequency band is needed to assess which frequency or combination of frequencies should be used for quantitative estimates of mysid abundance and for classification of targets in freshwater lakes. Our objectives in this paper are (*i*) to measure the frequency response and estimate TS of *Mysis relicta* at five frequencies (38, 120, 200, 430, and 710 kHz), (*ii*) to determine the detection range of mysids at each frequency, (*iii*) to compare the mysid frequency response with that of fish, and (*iv*) to compare the measured frequency response with predictions from a theoretical scattering model.

Materials and methods

Study area

The 172 km^2 Cayuga Lake (42°41′N, 76°41′W) is one of New York State's Finger Lakes. It is deep (maximum depth 132 m, mean depth 54 m) and mesotrophic and has an abundant mysid population. The most abundant fish in the lake is the alewife (*Alosa pseudoharengus*). Piscivores include several trout and salmon species, and there is a thriving fishery for these predators. Details on the lake can be found in Oglesby (1978).

Field collections

Acoustic data were collected during the night on 31 August 2006 using five frequencies (38, 120, 200, 430, and 710 kHz). The moon had set at the time of data collection. All transducers were suspended from a small boat and data were collected while drifting slowly over 85–95 m deep water. During the 3 h of data collection, the boat drifted approximately 1 km. For four of the five frequencies (38, 120, 200, and 710 kHz), Simrad EY60 units (Kongsberg Maritime AS, Horten, Norway) were mounted on one raft and pulse transmission was simultaneous at all frequencies. For the fifth frequency (430 kHz), a BioSonics DtX unit (BioSonics, Inc., Seattle, Washington) was mounted on the opposite side of the boat and operated independently of the other frequencies. (Specifications for all units are given in Table 1.) All acoustic data were collected with no onboard light as mysid acoustic backscattering decreases in light, probably an effect of changes in orientation associated with avoidance reactions to light (Gal et al. 1999*b*).

Mysids were sampled with a 1 m diameter, 3 m long, opening and closing net (1 mm mesh) from an adjacent boat. Duplicate samples were collected from depths of 15–0, 30–0, 40–0, and 60–40 m. All animals were preserved in the field and measured (tip of rostrum to cleft of the telson) and enumerated in the laboratory.

Temperature, dissolved oxygen (DO), chlorophyll *a* (chl *a*), and turbidity were measured with a Sea Bird profiler (Sea-Bird Electronics, Inc., Bellevue, Washington). Temperature profiles showed a well-defined thermocline at a depth of 15–18 m with an epilimnetic temperature of 21 °C and a hypolimnetic temperature of 5 °C. Chlorophyll values were around 3 μg·L^{-1} in the epilimnion, decreasing to close to 0 in the hypolimnion (Fig. 1). Oxygen levels were above 5 mg·L^{-1} throughout the water column, with a slight minimum at the thermocline. There was also an increase in turbidity at the thermocline, a slow increase in turbidity with depth through the hypolimnion with a more marked increase at 70 m depth. Most frequencies detected the turbidity increase at the thermocline and the 38 kHz also detected the increase in turbidity at 70 m depth (Fig. 1).

Analysis

All acoustic data was analyzed using Sonar5 (Balk and Lindem 2007).

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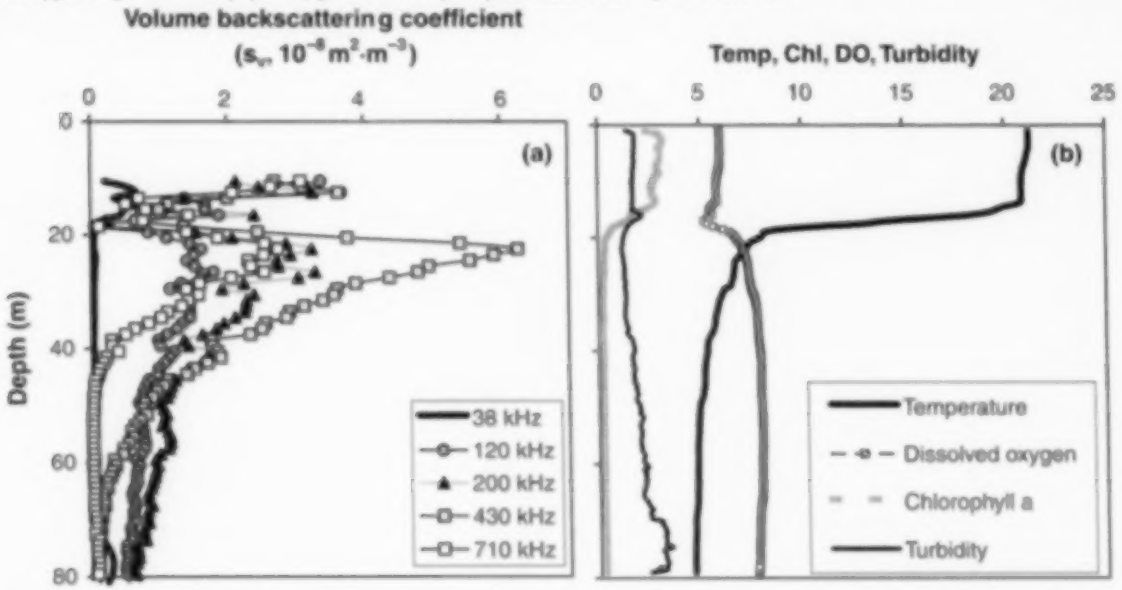
Fig. 1. Depth profiles of volume backscattering coefficient (s_v; $m^2 \cdot m^{-3}$ at five frequencies and environmental data (temperature (°C), dissolved oxygen ($mg \cdot L^{-1}$), chlorophyll *a* ($\mu g \cdot L^{-1}$), turbidity (nephelometric turbidity units, NTU)).



File preparation

Sound absorption and sound speed were calculated for each depth using the temperature profile and were adjusted dynamically with depth. This was particularly important for the high frequencies where absorption is a large source of sound attenuation and changes exponentially with temperature (Francois and Garrison 1982). For example, absorption for the 710 kHz unit is 108 $dB \cdot km^{-1}$ at the surface temperature of 21 °C and 194 $dB \cdot km^{-1}$ at the hypolimnetic temperature of 5 °C present on the night of sampling. Background noise was measured using passive recordings at each frequency and removed by subtraction from the data. The units were calibrated the same week (38, 120, and 200 kHz split-beam units) using standard targets, or calibrated by the manufacturer the following winter (the two single-beam units, 430 kHz at BioSonics and 710 kHz at Simrad). Calibration of both single-beam units showed stable performance over time (difference in the source level was 0.37 dB from three calibrations in 2004, 2005, and 2007 for the 430 kHz unit and difference in transducer gain was 0.15 dB for two calibrations in 2006 and 2007 for the 710 kHz unit). Calibration constants were adjusted as needed prior to the analysis. Depths of the transducers were set to correspond to the actual depth relative to the surface. Synchronization of the data recorded by the Simrad and the BioSonics systems was done in two steps. First, the time stamps in the data strings from the two units were adjusted to the same reference time (Greenwich). Second, for each ping recorded by the Simrad system, the closest ping in time was selected from the BioSonics data. This gave a synchronized file set with five channels where the 430 kHz pings never deviated more than ±0.38 s from the others.

Fish echo removal

The contribution of fish to the volume back-scattering coefficient (s_v, $m^2 \cdot m^{-3}$) was removed by identifying fish on the 38 kHz data and constructing a mask to exclude those data from the analysis. Excluded sections were not considered when calculating average s_v. This is equivalent to assuming the same mysid density in areas with and without fish, a reasonable assumption given the relatively large pulse volume in the mysid layer. With a 0.5 ms pulse duration, the sampling volume is 1.7 m^3 at a depth of 20 m and 7.0 m^3 at 40 m. Because the 38, 120, 200, and 710 kHz transducers were mounted close to each other and had similar beam widths (Table 1), there was high overlap in insonified water at the depth of the mysid layer (20–40 m). A mask derived from the 38 kHz data was applied to these frequencies, while a separate mask had to be generated and applied to the 430 kHz data because this transducer's beam did not overlap the others. A cross-filter detector (Balk and Lindem 2000) was applied to detect fish traces and produce the fish removal masks consisting of samples within the fish traces. The detector operates with a set of image analysis commands. This includes a median filter (3 × 3, an edge-preserving, low-pass filter that removes noise while preserving the edges of the fish traces), a cross filter (foreground 3 × 3, background 55 × 1, offset 6 dB, produces an adaptive threshold just above the mysid and background reverberation), and a mean filter (1 × 5, "fattens" the traces by smearing out echo energy from the detected fish into surrounding echo samples in a similar way as a growing operator). The mean filter was applied to increase the size of the fish trace and subsequent mask to avoid including fish echoes from the beam periphery and to account for minor deviance in beam overlap when applying the mask to the echograms from the other transducers. The masks were scrutinized and edited manually before they were applied to avoid any samples from fish contributing to the mysid analysis.

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Frequency response

The s_v in the mysid layer was calculated for ten 300-ping segments from the 20–30 m depth layer. Deeper water was not included because of low signal-to-noise ratio at 710 kHz. For each segment, the s_v value at 430 kHz was calculated and all s_v values for other frequencies were related to that value. The 430 kHz data had the highest s_v values in all segments. We used these 10 segments as independent measures of the frequency response and calculated standard errors from these values. Fish traces were common above the thermocline (15 m to the surface), and we used data from all five frequencies with fish echoes included to represent the frequency response of fish and other targets in water depths of 10–20 m. We limited this analysis to water deeper than 10 m to avoid the near field (two times the theoretical near field is 5.5 m) of the large 38 kHz transducer.

Target strength

Only a single mysid was caught in the two 15 m to surface net tows. Therefore, we estimated mysid TS for each frequency by comparisons of s_v data with mysid density estimated by the vertical net tows from the mysid layer (from 20 m to 30 or 40 m depth). Ranges of TS estimates were obtained by calculating a TS value given the range of densities obtained in the four net tows through the mysid layer. This comparison assumes a 100% net efficiency. There was no wind at the time, making the tows almost vertical. Although filtering efficiency is likely close to 100% given the relatively large mesh size of these nets, some net avoidance by mysids may occur. Thus, sampled densities may be biased low and therefore mysid TS may be biased high.

Detection range

Rudstam et al. (2008) defined the detection range for mysids as the depth at which the signal-to-noise ratio from a mysid density of 1 animal·m^{-3} is 3 dB (a factor of two). This detection range depends on the TS of an average mysid (which is frequency-dependent, see Results and discussion), the noise level for each frequency, and sound spreading and absorption (which is also frequency-dependent; Table 1). We used the estimated TS of a mysid from Cayuga Lake and the measured noise levels to calculate the detection range for our analysis. We also calculated the detection range if the noise levels were –125 dB at 1 m depth (S_v domain), a value that is reasonable during standard surveys in the Great Lakes (Rudstam et al. 2008). In practice, the noise levels will vary among frequencies because of the unknown frequency response of ambient noise and differences in power output. Higher frequencies cannot be used at as high a power level as lower frequencies because of nonlinear acoustic propagation where energy is lost from the fundamental frequency into higher harmonics (Tichy et al. 2003).

Comparisons with theoretical scattering models

There are a number of scattering models that have been used for euphausiids and mysids in the literature (Stanton and Chu 2000; Lavery et al. 2002; Demer and Conti 2005). Gal et al. (1999*a*) used the average ray solution of the distorted-wave Born approximation derived by Stanton et al. (1993) and the parameters for a weakly scattering fluid-like bent cylinder to model the TS of *Mysis relicta* in Lake Ontario. This simplified solution applies to elongated, bent organisms with sound-scattering characteristics that deviate only slightly from their environment and exhibit a narrow Gaussian distribution around the normal and broadside incidences. The equation is (Stanton and Chu 2000, eq. 7, retaining their symbols):

$$\langle \sigma_{bs} \rangle_{L,\theta} = 2A_{ij}R_{12}^2 aL[1 - e^{-8k\alpha s_\theta^2} \cos(4ka + \mu)]$$

where $\langle \sigma_{bs} \rangle_{L,\theta}$ is the average backscattering cross section of the animal at length L and angle of orientation θ [TS = $10\log_{10}(\sigma_{bs})$]; A_{ij} is a shape factor equal to $0.1 \cdot s_\theta^{-1}$ for a bent cylinder, where s_θ is the standard deviation of the orientation angle (in radians); R_{12} is the reflection coefficient [$R_{12} = (gh - 1)(gh + 1)^{-1}$]; g is the density contrast between the animal and the water; h is the sound speed contrast between the animal and the water; a is the average radius of the animal; k is the wave number ($k = 2\pi \cdot \lambda^{-1}$), which we assumed equal in the animal and the water; λ is the wavelength; and μ is defined as [$\mu = -(\pi/2) - ka - (ka + 0.4)^{-1}$]. Stanton and Chu (2000) states that this formula is valid for $ka > 0.1$, which includes almost all animal size and frequency combinations of interest here (a 10 mm mysids has a radius of 0.5 mm, which result in a ka of 0.08 at 38 kHz). Although this equation is complex, it contains a limited number of unknowns, the product of g and h, the standard deviation of the average angle of orientation (s_θ), the radius a, which we measured for mysids to be 5% of the length, the standard deviation of the length s_L, and the average orientation θ. For the purpose of this paper, we will use the value 0.36 for s_L measured for *Mysis relicta* by Gal et al. (1999*a*) and an average dorsal aspect orientation and density and sound speed contrasts measured for krill (*Euphausia pacifica*) by Chu and Wiebe (2005). Chu and Wiebe (2005) measured a g of 1.0156 and an h of 1.021 for 25 mm euphausiids in salt water (also used by Lawson et al. (2006) for krill smaller than 25 mm). These contrasts were translated into contrasts of a mysid in freshwater by accounting for the differences in sound speed and density of salt water and freshwater at 4 °C. This assumes the same body composition of marine krill and freshwater mysids. The resulting values for mysid in freshwater are $g = 1.044$ and $h = 1.051$. We do not know of any measurements of g and h for zooplankton or mysids in freshwater. The final unknown parameter s_θ was estimated by minimizing the squared difference between our model estimate of σ_{bs} and the measured σ_{bs} at all five frequencies. We also compared the estimates using the original model parameters suggested by Stanton et al. (1993) for euphausiids and the model parameterization by Gal et al. (1999*a*) for *Mysis relicta* (Table 2). We refer the reader to Stanton and Chu (2000) for more details on the derivation of this model.

Results and discussion

Mysids were most abundant in the net tows from depths of 30 or 40 m to the surface (Table 3). Mysids were also present at lower densities in net tows from 60 to 40 m (the net was closed at 40 m depth in those tows). Only one mysid was caught in two net tows from 15 m to the surface. The lack of mysids in the top 15 m corresponded well with

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Table 2. Parameter values used for modeling mysid target strength using the Stanton et al. (1993) weakly scattering, fluid-like, bent-cylinder model.

Parameter	Stanton et al. (1993)	Gal et al. (1999*a*)	"Best-fit" model
g (density contrast between the animal and water)	1.06	1.07	1.044
h (sound speed contrast between the animal and water)	1.06	1.07	1.051
s (standard deviation of the length)	0.10	0.36	0.36
s_θ (standard deviation of orientation (radians/degrees))	0.52/30°	0.72/42°	0.475/27°

Note: Equations are in Stanton and Chu (2000, eq. 7) and in the Materials and methods; we retained the original symbols. Note that s_θ needs to be converted to radians for use in the equation. The average radius of a mysid was measured to be 5% of the length of the animal. g and h in the "best-fit" model are derived from Chu and Wiebe (2005).

Table 3. Mysid density and size distribution in Cayuga Lake, New York, on 31 August 2006.

Depth sampled	No. caught	Density (no.·m^{-3})	Average length (range) (mm)	% females of animals >12 mm
15–0 m	0	0	NA	NA
15–0 m	1	0.1	18	100
30–0 m	31	3.9	11.7 (5–18)	83
30–0 m	41	5.2	11.7 (5–18)	74
40–0 m	64	4.1	11.9 (5–18)	69
40–0 m	70	4.4	13.0 (5–21)	74
60–40 m	15	1.0	13.7 (5–18)	50
65–40 m	6	0.3	13.5 (9–20)	50
Mysis layer (20–40 m)	206	4.4	12.2 (5–21)	74

Note: Density for tows from 30 m or 40 m calculated assuming all mysids were caught below 20 m depth (the top of the acoustic mysid layer). The average density in the mysid layer is calculated as the average of densities obtained from 30 to 20 m and from 40 to 20 m.

the acoustic data, which indicated that the mysid layer was below 20 m (Figs. 1 and 2), starting below the thermocline, in line with expectations from other systems (Gal et al. 2006; Boscarino et al. 2007). Here we define the mysid layer based on the combined results of acoustics and net tows as water depth between 40 and 20 m. The water column density in the sampling area was 102 individuals·m^{-2} (sum of the mean density in the 60 to 40 m and the 40 m to surface tows; Table 3). Mysids ranged in length from 5 to 21 mm, and the length distribution showed three peaks. The 9–13 mm animals are likely mysids hatched in the spring of 2006, and the larger individuals are surviving adults from 2005 (Fig. 3). The mysids around 5 mm may represent a late-summer generation. The average size of mysids caught from 40 m depth was 12.5 mm and from 30 m depth was 11.7 mm (overall average of 12.2 mm; Table 3).

Acoustic echograms show abundant fish traces above the thermocline, a layer almost devoid of scattering below the thermocline, and a mysid scattering layer with higher s_v values between 20 and 30 m and decreasing at depth > 30 m (Figs. 1 and 2). Volume backscattering displayed in the figures decreased faster with depth at 430 kHz and 710 kHz than at 120 kHz and 200 kHz. At the higher frequencies, the signal is attenuated faster with depth because of higher sound absorption causing the signal to be below the noise level at a shorter range than for the lower frequencies. As noise levels are subtracted from the signal in our analysis, no backscattering below the noise level is shown in these figures (Fig. 2).

The frequency response of mysid s_v was calculated for the 20–30 m depth region, where there should be little loss of signal from mysids even at 710 kHz. This response shows a large increase from 38 to 120 kHz, a further smaller increase to 200 kHz, a peak at 430 kHz, and a decline to 710 kHz (Fig. 4). The S_v data from 430 kHz was 19.5 dB higher than from 38 kHz, 5.2 dB higher than from 120 kHz, 2.5 dB higher than from 200 kHz, and 3.5 dB higher than from 710 kHz. This pattern was consistent among segments. The range of variation in the relative values within the various frequencies was 1.3–2.2 dB (Fig. 4).

The TS of an individual mysid was estimated to be –99.4 dB at 38 kHz, –84.9 dB at 120 kHz, –82.4 dB at 200 kHz, –80.1 dB at 430 kHz, and –83.7 dB at 710 kHz (Table 4; Fig. 5). These TS estimates were based on comparisons of density and s_v values in depth layers 40–20m or 30–20m, dependent on depth of net tows. They are in good agreement with previous measurements reported in the literature. To allow comparisons of our values with literature data collected at different frequencies and mysid length, we plotted the reduced target strength (RTS = TS – 20·log$_{10}$(L)) against ka (Fig. 6). For *Mysis relicta*, Gal et al. (1999*a*) reported TS at 420 kHz ranging from –79 dB to –73.1 dB and Rudstam et al. (2008) reported TS at 120 kHz of –86.3 dB. A freshwater pelagic amphipod (*Macrohectopus branickii*) with similar size, shape, and behavior as *M. relicta* has a TS of –82 dB at 200 kHz (Rudstam et al. 1992*b*). For marine mysids at 420 kHz, reported TS values range from –82.9 to –72.9 dB, partly dependent on mysid length (Wiebe et al. 1990; Demer and Martin 1995). The largest difference between our measures and those in the literature is from our own earlier comparison between s_v and net tows at 420 kHz (Gal et al. 1999*a*). However, the scatter in our earlier data was relatively large, and we did not have the same fish removal filters available at the time, which may have biased the TS high.

It should be remembered that TS of an elongated target like a mysid is strongly dependent on orientation (McGehee et al. 1998). We know that acoustic backscattering decreases dramatically when a light is shined on the animals at night (Gal et al. 1999*b*; Rudstam et al. 2008). In the laboratory, we have observed that such light levels elicit an avoidance behavior causing the animals to quickly swim downwards with head first, thereby changing orientation relative to the acoustic beam. This can cause the mysid layer to disappear acoustically — the return becomes lower than the noise

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Fig. 2. Echograms (S_v domain) from five frequencies (38, 120, 200, 430, and 710 kHz) in Cayuga Lake, New York, on 31 August 2006. Noise has been subtracted from these data, which is the reason for the low echo levels at depth at the higher frequencies. The dB scale ranged from –90 dB (white) to –54 dB (dark red). Detection ranges for a mysid density of 1 animal·m^{-3} given the conditions during data collection were 36 m (710 kHz), 53 m (430 kHz), and over 100 m (38, 120, and 200 kHz). Even though the detection range is long for 38 kHz, the mysid scattering is below the –90 dB lower threshold applied in these figures.

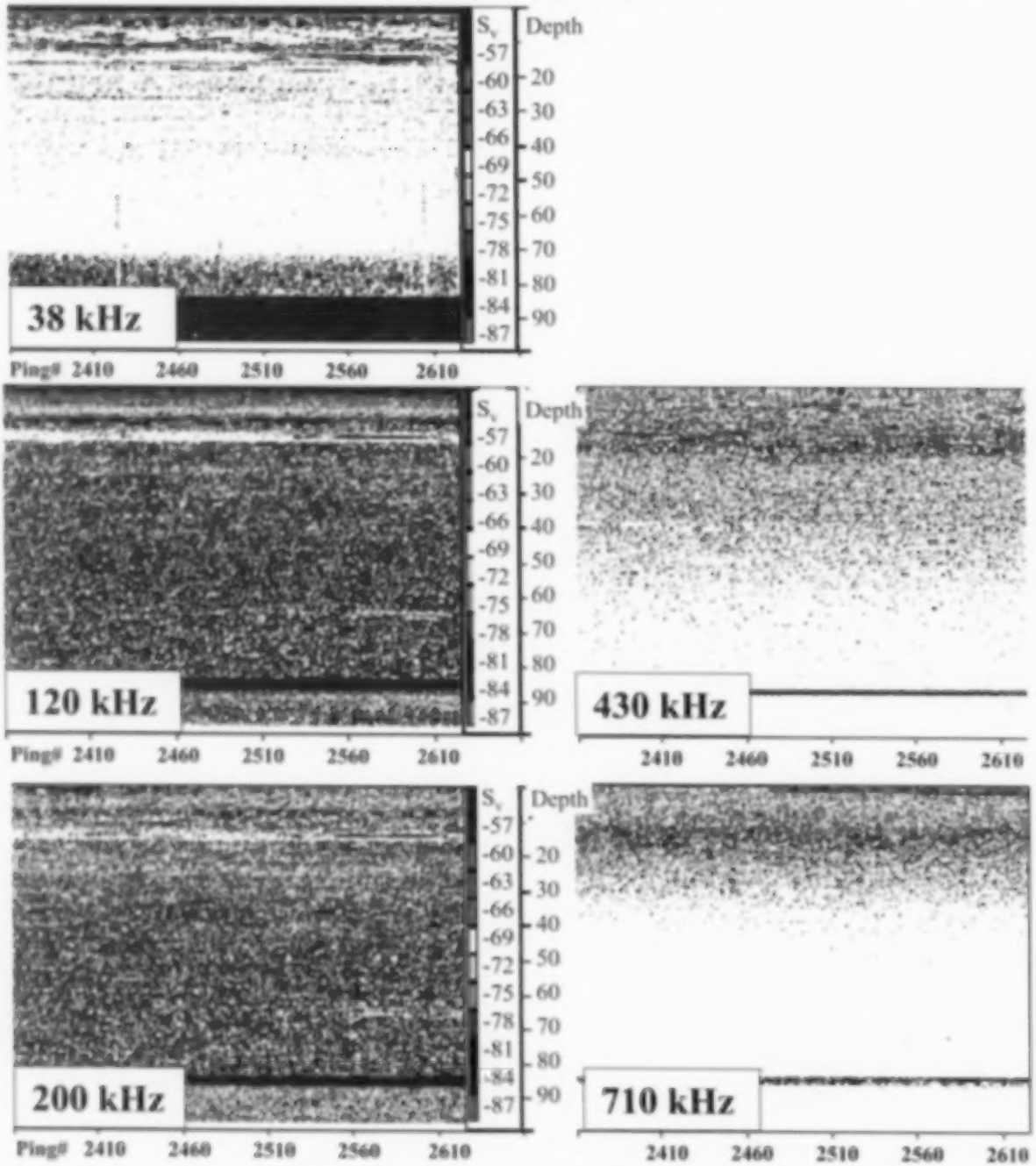

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Table 4. Mysis target strength (TS) at different frequencies in Cayuga Lake, New York, in the mysid layer (20–40 m).

Frequency (kHz)	TS (range) (dB)	Detection range (m) (–125 dB@1 m / ambient)	Gal (dB)	Stanton (dB)	Best-fit model (dB)
38	–99.4 (–99.9 to –98.7)	13 / 112	–97.28	–101.31	–98.66
120	–84.9 (–84.3 to –85.6)	67 / 283	–85.04	–86.07	–86.43
200	–82.4 (–81.6 to –82.9)	76 / 311	–80.89	–80.69	–82.27
430	–80.1 (–79.1 to –80.7)	58 / 53	–79.76	–78.84	–81.14
710	–83.7 (–82.6 to –84.6)	29 / 36	–80.29	–80.32	–81.68

Note: Average size of mysids in the samples was 12 mm. Range of TS is calculated from the range of density estimates in four vertical net tows. Theoretical predictions were obtained using the fluid-like, bent-cylinder model described in Stanton et al. (1993) and Stanton and Chu (2000, their eq. 7) using parameters in Table 2. The detection range is calculated both for a normal operation noise level at 1 m of –125 dB in the S_v domain and for the measured noise levels in Cayuga Lake during the low-noise conditions of the data collection (no engine, battery operation of sounders).

Fig. 3. Size distribution of mysids in the net hauls from Cayuga Lake, New York, on 31 August 2006. Total number of animals caught was 227.

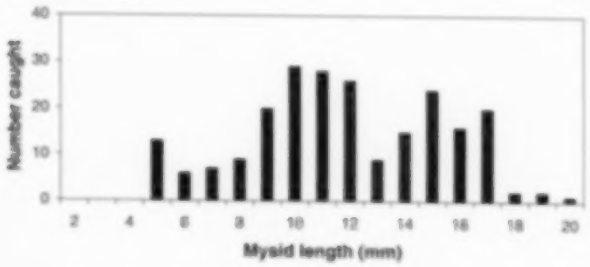
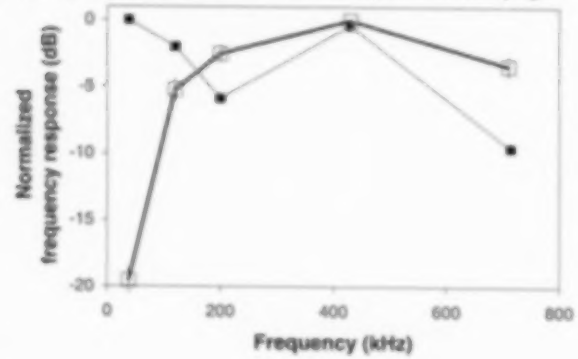
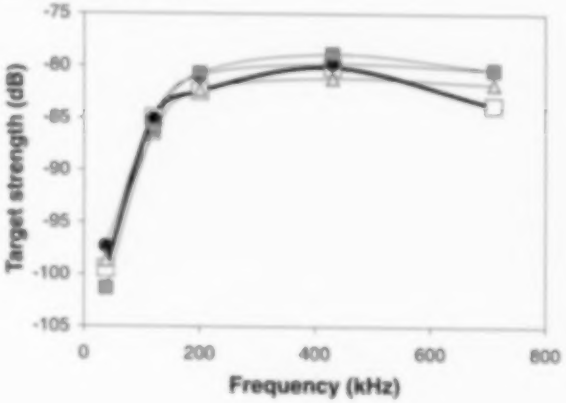


Fig. 4. Normalized frequency response of acoustic backscattering at 38, 120, 200, 430, and 710 kHz in Cayuga Lake, New York, in two depth layers dominated by fish (10–20 m; solid squares) and *Mysis* (20–40 m; open squares). Fish echoes were removed prior to determination of the frequency response for *Mysis*. The frequency response in the fish layer includes all targets. All values are related to the s_v value for the frequency with maximum return (38 kHz for the fish layer and 430 kHz for the mysid layer). The bars for the mysid layer represent the range observed in 10 segments of 300 pings.



flour. Similarly, the dorsal and ventral TS of euphausiids are 20–30 dB higher than the head-on or tail-on orientation (McGehee et al. 1998), making the acoustic estimates of krill highly dependent on their orientation in the field (Demer and Conti 2005). Although our laboratory observations suggest that mysids are swimming with a slight upwards tilt in dark conditions, we have no information on the

Fig. 5. Theoretical and observed frequency response of mysid target strength in Cayuga Lake. The three models are variations on the fluid-like, bent-cylinder model of Stanton et al. (1993) and include the Stanton parameterization for krill (shaded squares), the Gal parameterization for mysids (solid circles), and the best-fit model using updated density and sound contrasts (shaded triangles). Target strength (TS) calculated from the observed density is represented by open squares, range in Table 3. Parameter values for the models are in Table 2.



orientation of mysids in the lake and how variable such orientations are over time. Even so, our values should be applicable to a population swimming normally and feeding in the lower metalimnion and upper hypolimnion. However, it should be remembered that the TS values presented here are specific to the size structure of the population in Cayuga Lake and that the TS values include a bias associated with net avoidance of mysids because they are derived from comparison of s_v with net catches. In the future, it may be possible to use a theoretical scattering model directly to calculate mysid TS from the size distribution in the lake if the components of the scattering models are verified experimentally.

Detection ranges are calculated based on these TS values and both ambient measured noise with the five frequencies and assuming a standard noise level at 1 m depth of –125 dB (S_v domain). The detection range is very deep, over 200 m, at 120 and 200 kHz, whereas the detection range is shorter at 38 kHz because of the lower mysid TS and at 430 and 710 kHz because of higher

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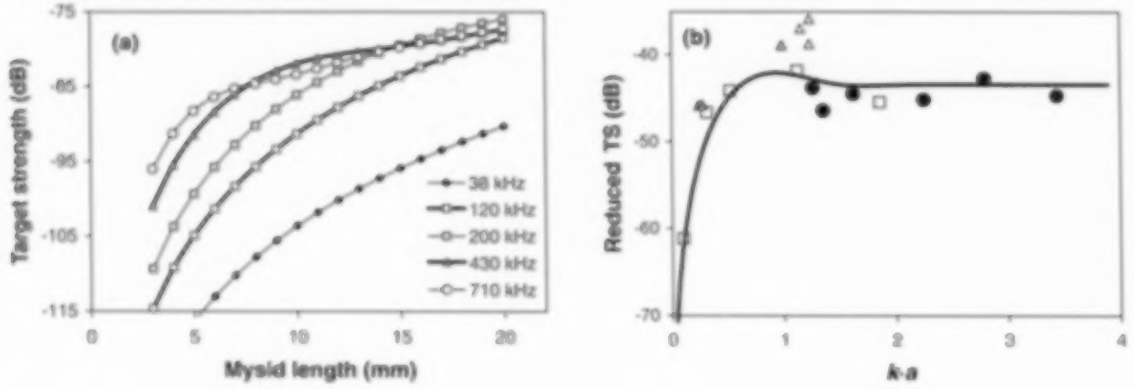
Fig. 6. (*a*) Predictions of mysid target strength (TS) as a function of animal length and frequency for the best-fit parameterization of Stanton's fluid-like, bent-cylinder model (see Materials and methods for equation, parameters in Table 2). (*b*) The same model predictions but plotted as reduced TS (RTS = TS – 20·log$_{10}$(*L*), where *L* is total mysid length (m)) against *ka* (*k* is the wave number and *a* the average radius of the mysid). In *b*, the first peak in the model predictions appears at a *ka* of approximately 1. Data points are from measurements from this study (open squares; five frequencies), Gal et al. (1999*a*) (open triangles; 420 kHz), Rudstam et al. (2008, shaded triangles; 123 and 430 kHz), and three species of marine mysids plotted as the end points of the range of length and TS used in the measurements (from Wiebe et al. (1990) and Demer and Martin (1995); solid circles).



sound absorption. We therefore recommend using either 120 or 200 kHz for estimating mysids in deep lakes. Note that we collected data while drifting and with the unit powered by car batteries, thus there was no engine noise present in the data. Under normal survey conditions, it may be possible to operate with noise levels of –125 dB at 1 m (S_v domain). At such noise levels, we can expect a detection range of 60–70 m for the 120 and 200 kHz units (Table 4). We believe that most mysids are above this detection range during the summer when the moon is not full (Gal et al. 2006; Boscarino et al. 2007).

We calculated a theoretical frequency response of mysid TS given the length distribution observed in Cayuga Lake using three parameterizations of the theoretical scattering model of Stanton et al. (1993) and Stanton and Chu (2000, their eq. 7, also given in our Materials and methods). The first model is the original model parameterized for krill by Stanton et al. (1993). The second model represents the modification by Gal et al. (1999*a*) to better represent the TS of mysids in freshwater at 420 kHz. The third model is derived using the most recent measures of sound and density contrasts by Chu and Wiebe (2005), measures of the standard deviation of length for mysids of 0.36 from Gal et al. (1999*a*), and a best fit of this model given variable standard deviation of orientation (s_{bf}, see Materials and methods; Table 2). The s_{bf} in the Gal model was adjusted from 30° to 42° to correct for an error in the equation given for A_{ij} in that paper. The ranges of deviation from these models and observations were –0.1 to 3.4 dB for the adjusted Gal model, –1.8 to 3.4 dB for the Stanton model, and –1.5 to 2.0 dB for the best-fit model (Fig. 5). All three models predicted higher TS at 430 kHz than at the other four frequencies (Fig. 5; Table 4) and a large increase in TS from 38 to 200 kHz. The consistent increase in mysid TS between 38 and 430 kHz represents the first peak in the transition zone between Rayleigh scattering and geometric scattering (Medwin and Clay 1998; Lavery et al. 2002). Following the expectation of the Lavery et al. (2002) model (also based on the distorted-wave Born approximation), we expect the first peak in the transition zone between Rayleigh and geometric scattering to occur at a *ka* value of approximately 1, which would be at 389 kHz for a mysid with an average radius of 0.6 mm (12 mm animal) (Fig. 6). Interestingly, all three versions of the model predicted a smaller decline in TS at 710 kHz than observed, which may be associated with higher energy losses to attenuation at 710 kHz in the field. Note that the frequency response and average mysid TS are both sensitive to the size structure of the mysid population (Fig. 6) and to the exact shape and orientation of the animals (Lavery et al. 2002). Given the complex shape of these scatterers and unknown orientation in the field, theoretical results within 2 dB of observations can be considered acceptable and our best-fit model can be used as a first approximation for TS of mysids in the field. More complex models have been proposed (Stanton and Chu 2000; Lavery et al. 2002; Conti et al. 2005) and may give a better representation of the sound scattering from the animal, but they also require a better digitization of the animal's body shape than currently available for mysids and knowledge of the animal's orientation relative to the sound beam in the field. We also need actual measurements of sound speed and density contrasts between mysids and freshwater as body compositions may vary between a marine krill and a freshwater mysid.

The frequency response of fish above the thermocline was very different from the frequency response of mysids (Fig. 4). The fish layer showed the highest echo levels at 38 kHz, and the contrast between fish and mysids scattering was therefore particularly strong at that frequency. Adding this frequency to surveys in freshwater could help us separate fish and invertebrates. The differences in scattering at 38 and 120 kHz are useful in marine applications for separating krill and fish (Cochrane et al. 1991; Everson et al. 1993; Foote and Stanton 2000). Unfortunately, the 38 kHz

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transducer is large and difficult to include in a portable unit. Although likely not as useful as 38 kHz, a 50 or 70 kHz transducer is more portable and may provide sufficient contrast to the 120 or 200 kHz units to separate fish and mysids.

We do believe that the increase in observed mysid TS between 120 or 200 kHz and 430 kHz is real and not due to the differences in sounder manufacturer or fish removal methods. The fish removal filter applied was conservative in that most fish echoes should have been removed from the analysis. Therefore, our mysid estimates should not be biased much higher by inclusion of fish scattering. Calibration can be less accurate with single-beam than split-beam units because information on calibration sphere position in the acoustic beam is lacking, but the calibrations of the two single-beam units were confirmed by the manufacturers during the winter of 2006–2007. Further, additional data collected with different 120 kHz and 430 kHz transducers in Lake Ontario show similar increases in s_v at 430 kHz compared with at 120 kHz in the mysid layer (TS of –78.3 dB at 430 kHz and –85.2 dB at 120 kHz; L. Rudstam, unpublished data from 2005). We did not expect the frequency response to increase from 200 kHz to 430 kHz in the fish layer, but this may be due to orientation of the fish or inclusions of other epilimnetic targets such as zooplankton or bluegreen algae with higher volume backscattering at 430 kHz than at the lower frequencies.

Areal mysid densities in Cayuga Lake were around 100 individuals·m^{-2}, which is similar to densities in Lake Ontario and to estimates in Cayuga Lake in the 1990s and 2000s (Johannsson et al. 2003; Rudstam et al. 2008). The observed distribution is consistent with a model predicting the distribution of these animals in temperature gradients based on laboratory results (Boscarino et al. 2007). Mysids are important predators in most lakes where they occur but are limited in their impact on the upper pelagic food web during the summer stratified period because of their avoidance of epilimnetic temperatures (Beeton and Bowers 1982; Gal et al. 2006). Both the total abundance and depth distribution of mysids are important for understanding their role in the pelagic food web, and we have shown that both can be obtained with acoustics frequencies often used in fisheries surveys in freshwater lakes (120–200 kHz). However, it would be helpful to also include a lower frequency in freshwater fisheries surveys in lakes with mysids (38 kHz or 70 kHz) to improve our ability to differentiate between acoustic returns from fish and mysids (see also Jurvelius et al. 2008). Higher frequencies, such as 430 kHz and 710 kHz, add little to our ability to discriminate between mysid and fish but could be useful for measures of smaller zooplankton.

Acknowledgments

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A mitochondrial multigene approach contributing to the systematics of the brook and river lampreys and the phylogenetic position of *Eudontomyzon mariae*

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Abstract: The evolution of nonparasitic brook lamprey forms and their relationship to the morphological similar parasitic river lampreys are long-standing questions in lamprey research. The present study examines the utility of 11 mitochondrial gene segments (a total of 3800 base pairs) to discriminate the closely related members of the European species pair *Lampetra fluviatilis* and *Lampetra planeri*. The intent was to identify fast-evolving genes in the mtDNA that might allow a discrimination of pair members. Therefore, the noncoding regions as the fastest evolving gene segments of animal mtDNA were also included. The results revealed that in all investigated loci, *L. fluviatilis* and *L. planeri* were genetically indistinguishable, indicating either a very recent divergence event or ongoing gene flow between both species. The position of the Ukrainian brook lamprey, *Eudontomyzon mariae*, and the phylogentic relationships within the Lampetrini was inferred from partial cytochrome *b* by adding available sequences from GenBank to the data set. *Eudontomyzon mariae* formed a monophyletic cluster with *Lampetra aegyptera* and the European species pair. A reintegration of *E. mariae* in the genus *Lampetra* is therefore recommended.

Résumé : L'évolution des formes de lamproies de ruisseau non parasites et leur relation avec les lamproies de rivière parasites de morphologie semblable sont des questions d'intérêt depuis longtemps dans la recherche sur les lamproies. Notre étude examine l'utilité de 11 segments de gènes mitochondriaux (un total de 3800 paires de bases) pour distinguer les deux espèces fortement apparentées de la paire de lamproies européennes *Lampetra fluviatilis* et *Lampetra planeri*. Notre objectif est d'identifier des gènes de l'ADNmt à évolution rapide qui puissent permettre de séparer les membres de cette paire. Nous avons donc inclus les régions non codantes comme les segments de gènes à évolution la plus rapide dans l'ADN mitochondrial chez les animaux. Nos résultats montrent que *L. fluviatilis* et *L. planeri* ne peuvent être distingués génétiquement par aucun des locus étudiés, ce qui indique soit un événement de divergence très récent ou un flux génétique persistant entre les deux espèces. Nous avons déduit la position de la lamproie de ruisseau ukrainienne, *Eudontomyzon mariae*, et les relations phylogénétiques au sein des Lampetrini de l'analyse partielle du gène du cytochrome *b* en ajoutant des séquences disponibles dans GenBank à la banque de données. *Eudontomyzon mariae* forme un groupe monophylétique avec *Lampetra aegyptera* et la paire d'espèces européennes. Nous recommandons donc de réintégrer *E. mariae* dans le genre *Lampetra*.

[Traduit par la Rédaction]

Introduction

The repeated emergence of nonparasitic brook lamprey forms is a phenomenon very well known from the Petromyzontiformes. The evolution of these forms and their relationship to the morphological similar, parasitic river lampreys are long-standing questions in lamprey research. Nearly one-half of the almost 40 extant lamprey species known worldwide is represented by purely freshwater, nonparasitic brook lampreys. The other taxa exhibit an anadromous life history that includes a larval phase of 4 to 5 years. After metamorphosis, these species migrate downstream to sea for parasitic feeding on various fish species. The subsequent spawning phase takes place after upstream migration in freshwater streams. Among the parasitic-feeding species, there are several landlocked forms that stay in fresh water during their whole life (e.g., Hubbs and Potter 1971; Maitland et al. 1994; Goodwin et al. 2006).

In contrast with that, the life cycle of brook lampreys lacks the parasitic feeding phase, and the larval phase is prolonged to 6 to 8 years. Brook lampreys do not feed anymore as adults. Both forms, parasitic and nonparasitic, are semelparous and die after spawning (Hardisty 1986*a*; Holčík 1986).

In many cases, the nonparasitic and parasitic lamprey species are morphologically very similar, and they are called "paired species" because of their close relatedness (Zanandrea 1959). In fact, as there is often more than one brook lamprey that is closely related to a parasitic species, the

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term "satellite species" is preferred (Vladykov and Kott 1979*a*).

It is widely accepted that the brook lamprey evolved from a parasitic ancestor similar to the closely related extant parasitic species (Zanandrea 1959; Hubbs and Potter 1971; Hardisty 1986*a*). Often the geographical distributions of the parasitic and nonparasitic forms largely overlap. An allopatric distribution of both forms either might be an indication for a relict occurrence of a formerly wider-distributed brook lamprey species or might be the result of a local extinction of the ancestral parasitic form (Hardisty and Potter 1971; Hardisty 1986*a*).

Traditionally, morphological characters such as dentition patterns, velar tentacles, number of myomers, pigmentation, and body size are used in lamprey taxonomy (Hardisty and Potter 1971; Vladykov and Kott 1976). The larvae of paired species are morphologically very similar. Satellite species are separated from their related parasitic species by the considerably smaller adult body size, a lower number of trunk myomers, a reduced fecundity, and a lower sharpness and general reduction in dentition (Hardisty and Potter 1971; Hardisty 1986*a*). Paired species are also characterized by the absence or low genetic divergence found, for example, in allozyme electrophoresis (Beamish and Withler 1986; Schreiber and Engelhorn 1998; Yamazaki and Goto 1998) and in markers of the mitochondrial DNA (mtDNA) (e.g., Tagliavini et al. 1994; Docker et al. 1999; Espanhol et al. 2007).

The present study is a contribution to the molecular investigations of the differentiation of brook and river lampreys using a mitochondrial multigene approach. We studied the European lamprey species pair consisting of the river lamprey, *Lampetra fluviatilis* (Linnaeus, 1758), and the brook lamprey, *Lampetra planeri* (Bloch, 1784), as an example for the evolution of species pairs. The pair members occur sympatrically throughout Europe in rivers draining into the Baltic Sea and North Sea, Atlantic, and western Mediterranean (Hardisty 1986*b*, 1986*c*). Our aim was to screen the mitochondrial genome to identify fast-evolving genes and to extend the current available genetic data for lampreys by characterizing the degree of variation of several new mitochondrial genes. In addition to the widely used protein-coding genes (e.g., Tagliavini et al. 1994; Docker et al. 1999; Espanhol et al. 2007), we sequenced the noncoding regions that are known for their rapid rate of evolution and that are therefore said to be useful for studying the relationship between closely related species (e.g., Hoelzel et al. 1991; Meyer 1993; Lee et al. 1995). Furthermore, we add new genetic data for the European lamprey species pair from localities (e.g., Baltic Sea drainage system) that were not studied so far in DNA-based investigations by comparing adult individuals of both species that were caught in the same river system.

Another objective was to examine the phylogenetic relationship of the Ukrainian brook lamprey, *Eudontomyzon mariae* (Berg, 1931), within the Petromyzontidae. This species is the most widespread of the genus and is found in the watersheds of the Baltic, Black, Mediteranean, and Azov seas (Renaud 1982; Holčík 1986). Its distribution range partially overlaps that of *L. planeri*, for example, in the upper tributaries of the rivers Neman and Vistula (Baltic Sea drainage system), the Danube River, and the upper and middle part of the Volga river basin (Zhukov 1965; Rembiszewski 1968; Levin and Holčík 2006). The position of this taxon, as well as of the genera *Lethenteron* and *Entosphenus*, is controversially debated (Vladykov and Kott 1979*b*; Potter 1980; Gill et al. 2003). In the studies published so far, the genus *Eudontomyzon* Regan 1911 was either neglected or the length of the sequence used was inappopriate to allow reliable conclusions (Tagliavini et al. 1996; Docker et al. 1999; Yamazaki et al. 2006).

Materials and methods

Taxon samples and DNA methods

To examine the genetic differentiation and relationship of the species pair *Lampetra fluviatilis* and *Lampetra planeri*, we sequenced 3800 base pairs from 11 gene segments of the mtDNA (Table 1). We used only adult individuals because of the fact that it is nearly impossible to reliably discriminate the larvae of both species using morphological characters. In the case of *L. fluviatilis* and *L. planeri*, an individual from one species originated from the same river system as an individual from the other species (Table 1). *Eudontomyzon mariae* (ammocoete) and *Petromyzon marinus* were included in the analysis as sister group and outgroup, respectively. For inferring the position of *E. mariae* within the Lampetrini, a part of the cytochrome *b* was used.

Total DNA was extracted from ethanol-preserved tissue by a silica gel-based spin column procedure according to the protocol of the manufacturer (High Pure PCR Template Preparation Kit, Boehringer Mannheim; E.Z.N.A. Tissue DNA Kit II, PEQLAB Biotechnology GmbH). Primers for polymerase chain reaction (PCR) (Table 2) were taken from different sources (Kocher et al. 1989; Pääbo et al. 1991; Meyer 1993) and optimized according to the published mitochondrial sequence of *P. marinus* (Lee and Kocher 1995; U11880). Binding sites of primers are shown in Fig. 1. Amplification of mtDNA fragments was carried out in 50 µL reactions containing 3 µL DNA template, reaction buffer (Genecraft), 3.0 or 4.0 mmol·L^{-1} $MgCl_2$ (Table 2), 250 µmol·L^{-1} of each dNTP, 10 pmol of each primer, and 0.25 U *Taq* polymerase (1 U = 16.67 nkat) (Amplitaq, Perkin Elmer; BioTherm, Genecraft). Additionally, the *Taq* PCR Master Mix Kit (Qiagen) was used with the same final concentrations of primers and $MgCl_2$. For PCR, either the Air Thermo Cycler (IDAHO Technology) or the PTC 100 (MJ Research) was used. PCR temperature profile for the amplification consisted of the following steps: initial denaturation at 94 °C for 1 min; 38 cycles of 5 s (Air Thermo Cycler) or 30 s (PTC 100) at 94 °C, 5 s (Air Thermo Cycler) or 30 s (PTC 100) at 50 or 60 °C, and 20 s (Air Thermo Cycler) or 1 min (PTC 100) at 72 °C; and 5 min at 72 °C (PTC 100).

PCR products were extracted from agarose gels using commercial kits (High Pure PCR Product Purification Kit, Boehringer Mannheim; E.Z.N.A Cycle-Pure Kit, PEQLAB Biotechnology GmbH), followed by precipitation (1 volume PCR product + 1 volume isopropanol + 0.1 volume ammoniumacetate). Sometimes we had difficulties to directly sequence particular PCR products; therefore, we cloned the PCR products of COI, Cyt *b*, ND3, noncoding region (NC)

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Table 1. Sampling information and GenBank accession numbers of the taxa included in the study.

Species (n; stage[a])	Haplotype	Sampling site; sampling date; source	16S rRNA	COI	Cyt *b*	ND3	NC
Watershed: Warnow River							
L. planeri (1; A)	01	Nebel Stream (near Kirch Rosin) D; May 1994; R. Lemcke	EU404072	EU404065	EU404061	EU404053	EU404082
L. fluviatilis (1; A)	04	Beke Stream D; April 1995; R. Lemcke	EU404071	EU404066	EU404059	EU404050	EU404079
Watershed: Elbe River							
L. planeri (1; A)	16	Stepenitz Stream D; June 1994; R. Lemcke	EU404072	EU404064	EU404061	EU404052	EU404084
L. fluviatilis (1; A)	85	Elbe River (near Brunsbüttel) D; October 1996; R. Lemcke	EU404072	EU404066	EU404060	EU404051	EU404080
P. marinus (1; A)	53	Elbe River (near Gorleben) D; June 1995; R. Lemcke	EU404075	EU404070	EU404057	EU404056	EU404085
Drainage: Baltic Sea							
L. planeri (1; A)	08	Althofener Bach D; May 1994; R. Lemcke	EU404072	EU404066	EU404061	EU404054	EU404083
L. fluviatilis (1; A)	91	Baltic Sea (near Barth) D; December 1995; R. Lemcke	EU404072	EU404066	EU404060	EU404051	EU404081
Watershed: Danube River							
E. mariae (2; Q)	41	Gail River A; February 1997; T. Friedrl	EU404076	EU404068	EU404063	EU404049	EU404077
	52		EU404076	EU404067	EU404062	EU404048	EU404078
Watershed: Rhine River							
P. marinus (1; A)	71	Rhine River (near Nuklear Power Station Philippsburg) D; March 1995; R. Engelhorn and A. Schreiber	EU404074	EU404069	EU404058	EU404055	EU404086

Note: COI, cytochrome *c* oxidase I; Cyt *b*, cytochrome *b*; ND3, NADH dehydrogenase subunit 3; NC, noncoding region.

[a]A, adult; Q, ammocoete.

Table 2. Primer sequences, fragment length, and information about polymerase chain reaction (PCR) conditions.

Fragment (length)[a]	PCR reaction (temperature, [Mg_2])	Primer	Reference
A1: ND6 + NCI + tRNA-Thr[b]	50 °C, 4 mmol·L^{-1}	LLamp15133[c]: 5′-TACCCGACCTCCAAGAACTTCAGG-3′	This study
(877 bp)		HLamp15967[c]: 5′-TCTACAACGGTGGTTTTTCA-3′	
A2: tRNA-Thr + tRNA-Glu + NCII[b]	50 °C, 4 mmol·L^{-1}	LLamp15898[c]: 5′-AAAGCACTGGTCTTGTAACC-3′	This study
(431 bp)		HLamp88[c]: 5′-TTTCATCAGGCCGAGATGTT-3′	
B: Cyt *b*	50 °C, 4 mmol·L^{-1}	LLamp107[c]: 5′-TGCTAACATCTCGGCCTGATGAAA-3′	This study
(756 bp)		HLamp814[c]: 5′-AATAGAAAGTATCATTCTGGTTTAAT-3′	
C: 16S	50 °C, 4 mmol·L^{-1}	16Sar: 5′-CGCCTGTTTATCAAAAACAT-3′	Kessing et al. 1989
(590 bp)		16Sbr: 5′-CCGGTCTGAACTCAGATCACGT-3′	
D: COI + tRNA-Ser + tRNA-Asp	50 °C, 3 mmol·L^{-1}	LLamp7292[c]: 5′-CCTGCAGGAGGAGGAGACCC-3′	This study
(1043 bp)		HLamp8298[c]: 5′-TTGAGCTTGTTGTGCCAT-3′	
E: ND3	60 °C, 3 mmol·L^{-1}	ND3ar: 5′-CACTTAATATAGTTGGCCTCCAACCA-3′	This study
(465 bp)		ND3br: 5′-GTTCAGGATTAATTGAGCCGAAATCA-3′	

Note: For primer binding sites, see Fig. 1.

[a]Calculated from the genome sequence of *Petromyzon marinus* (U11880; Lee and Kocher 1995).

[b]Noncoding region was amplified in two overlapping fragments.

[c]The number gives the 3′- binding site in the genome sequence of *Petromyzon marinus* (U11880; Lee and Kocher 1995).

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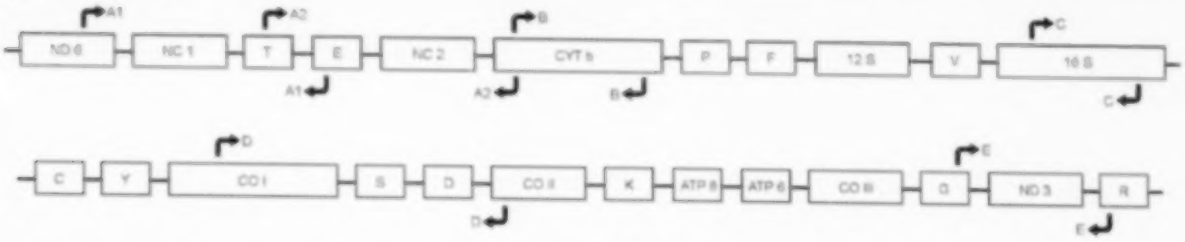
Fig. 1. Partial mitochondrial genome of *Petromyzon marinus* (U11880; Lee and Kocher 1995) showing the primer binding sites (arrows) and amplified fragments.



in pGEM-T vektor (Promega). The plasmids were isolated using the GFX Micro Plasmid Prep Kit (Amersham Pharmacia Biotech).

Both strands of every mtDNA segment were sequenced using primers labeled with IRD-800 (IRD-5′-16S ar, IRD-5′-16S; pUC/M13-univ(–21) IRD-5′-TGTAAAACGACGGCCAGT-3′; and pUC/M13-rev(-29) IRD-5′-CAGGAAACAGCTATGACC-3′ (for cloned sequences)) and a reagent kit from Epicentre Technology (Sanger et al. 1977). The sequences were electrophoresed and automatically analysed on 6% acrylamide–urea gels in Tris–borate–ethylenediamine–tetraacetate buffer (27 mmol·L^{-1}, pH 8.3) using an automatic sequencer (LICOR 4200; MWG Biotech). Alternatively, sequences were obtained by using the CEQ Dye Terminator cycle sequencing quick start kit (Beckman Coulter) according to the manufacturer's protocol and electrophoresed on an automated DNA sequencer (CEQ 8000; Beckman Coulter).

Data partitions and phylogenetic analysis

DNA sequences were aligned using BioEdit (Hall 1999) and controlled by eye. Phylogenetic analyses were performed using PAUP* version 4.0 b10 (Swofford 2000), TREEFINDER (Jobb et al. 2004) and MRBAYES 3.1.1 (Huelsenbeck and Ronquist 2001). All haplotypes were submitted to GenBank (accession No. EU404048-86; Table 1).

The study of mtDNA involved the following sets of analyses. First, each gene segment was analyzed separately. Then, two combined data sets were created: one including all mitochondrial gene segments and another with all gene segments except the noncoding region. *Petromyzon marinus* was used as outgroup taxon. The incongruence length difference (ILD) test (Farris et al. 1994, 1995) was used to test for heterogeneity between data partitions between mtDNA data sets. The ILD showed no significant incongruence between all partitions tested ($p > 0.03$), so we combined the data sets. For the analysis of the phylogenetic position of *E. mariae* within the Lampetrini, we combined our data from the Cyt *b* gene fragment with available sequences from GenBank (Supplemental Table S1, available online from NRC Data Depository[2]). *Mordacia mordax* was used as outgroup taxon. For convenience, we used the nomenclature and generic designations of Vladykov and Kott (1979*b*) and Renaud (1997). Homogeneity of base composition across ingroup taxa was assessed using the χ^2 test implemented in PAUP*.

To examine the variability of the gene segments and to find the appropriate weighting for the phylogenetic analysis, sliding-window analyses were performed (Pesole et al. 1992; Sturmbauer and Meyer 1992). The percentage of variation within windows of nine bases was determined with three bases overlap. The genetic variation was expressed as percentage of 27 possible base substitutions in one window of nine bases. Three classes of variation were defined: low-variable, 0%–20%; high-variable, 20%–40%; very high-variable, >40%. The transition–transversion (TS/TV) ratios were determined for each of these regions separately and, when required, translated into appropriate weights for parsimony analysis of the protein-coding gene data sets (COI, Cyt *b*, ND3).

For phylogenetic tree reconstruction, we used neighbor-joining (NJ), maximum parsimony (MP), maximum likelihood (ML), and Bayesian analysis (BA). For NJ, ML, and BA analysis, best-fit models and parameters for the different data sets were specified by the hLRT criterion in MrModeltest 2.2 (Nylander 2004; Table 3). Unweighted and weighted MP analyses were performed under the heuristic search option (100 replicates) with random addition of taxa, ACCTRAN, and TBR branch-swapping options. In the analysis of the 16S, the ambiguous alignment position 446 was excluded from analysis. In the tRNA-Asp gene segment (position 986 of COI alignment), the gaps were assigned as fifth character. In the noncoding regions, gaps of more than two base pairs were assigned as one substitution. The noncoding region revealed in three individuals (*L. fluviatilis* 91, *P. marinus* 53, and *P. marinus* 71) intra- as well as inter-individual length differences. These differences between base pairs 418 and 506 and between 1078 and 1213 of the alignment consisted of repeated sequence motifs that might originate from DNA slippage during replication (Lee and Kocher 1995). In the phylogenetic analysis, only the shortest sequences of the affected individuals was included.

Equally parsimonious trees were summarized using strict or 50% majority rule consensus. The robustness of the phylogenies obtained by ML, MP, and NJ were tested by bootstrap analysis (Felsenstein 1985) with 1000 replications. BA was conducted for each data set running four chains for 5 000 000 generations. Trees were sampled every 100 generations, and majority rule consensus trees were computed with 5000 trees excluded as the burn-in.

[2] Supplementary data for this article are available on the journal Web site (cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 3871. For more information on obtaining material refer to cisti-icist.nrc-cnrc.gc.ca/cms/unpub_e.html.

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Table 3. Summary of phylogenetic analyses.

	16S	Cyt *b*	ND3	COI	NC	Combined	Cyt *b* GenBank
No. of taxa	12	12	12	12	15	12	49
No. of haplotypes							
Ingroup	3	6	7	6	9	9	28
Outgroup	3	3	3	3	3	3	1
Aligned sequence length	464	813	351	1005	1249	3790	384
No. of variable, uninformative characters	5	10	10	8	28	58	48
No. of parsimony-informative characters	11	120	61	126	138	460	98
No. of MP trees	1	4	2	1	1	5	18
Length of MP trees	16	136	75	139	209	581	272
CI (all characters)	1.0	1.0	0.99	0.99	0.88	0.94	0.65
CI (excluding uninformative characters)	1.0	1.0	0.98	0.98	0.85	0.93	0.57
RI	1.0	1.0	0.99	0.99	0.91	0.97	0.83
RC	1.0	1.0	0.98	0.98	0.82	0.91	0.54
TS/TV ratio	—	3	3	4	—	—	6
Model of sequence evolution	F81	HKY85	HKY85	GTR	GTR	GTR	GTR
G	—	—	—	—	0.2557	0.4806	0.2566

Note: MP, most parsimonious; CI, consistency index; RI, retention index; RC, rescaled consistency index; TS/TV ratio, transition–transversion ratio; F81, Felsenstein model (Felsenstein 1981); HKY, Hasegawa–Kishino–Yano model (Hasegawa et al. 1985); GTR, general time-reversible model (Yang 1994); *G*, gamma distribution.

Results

Sequences, alignments, haplotypes, and patterns of divergence

The genetic distances between *L. fluviatilis* and *L. planeri* were in all loci at least as high as within one species and ranged from 0% (16S, COI) to 1.2% (ND3). The distances between *Lampetra* on the one hand and *E. mariae* and *P. marinus* on the other ranged from 0.9% (16S) to 8.2% (ND3) and 2.4% (16S) to 19.6% (ND3), respectively. *Eudontomyzon mariae* differed from *P. marinus* by 2.4% (16S) to 16.5% (ND3). The χ^2 test of homogeneity of base frequencies across taxa in all loci resulted in no significant *p* values ($p = 1.0$).

The genetic variability compared by a sliding-window analysis differed from gene to gene but was overall low in all data sets (as shown in Fig. 2). The number of substitutions was lowest in the 16S data set and highest in ND3, which is the fastest evolving of the analysed genes (16S < Cytb < COI < ND3). In the data set of the noncodong region, there were conservative stretches as well as regions with higher variation. One highly variable region was visible in the 5′ end of noncoding region I where the variability exceeded 30%.

The combined data set of all mitochondrial loci was represented by 12 haplotypes. Like in the separate loci, the genetic distances between *L. fluviatilis* and *L. planeri* did not exceed that of within-species distances (0.2%–0.4%). Both *Lampetra* species differed from *Petromyzon* and *Eudontomyzon* by up to 11.3% and 4.3%, respectively. *Eudontomyzon* was separated from *Petromyzon* by 11.3%. In the Cyt *b* data set, including GenBank data (Supplemental Table S1[2]), the pairwise distances within the Lampetrini (Kimura two-parameter distances, Kimura 1980) varied from 0.0% to 10.0%, and the distances to the sister clade formed by *Petromyzon* and *Ichthyomyzon* ranged from 13.4% to 17.3%, respectively.

Phylogenetic analysis of separate and combined mtDNA loci

A summary of the results of the phylogenetic analyses is provided (Table 3). The phylogenetic trees for each locus resolved the same topology independently of the employed reconstruction method (NJ, MP, ML, BA). Differences in branching patterns of trees between loci were due to different numbers of haplotypes. No data set or method found a distinct split between *L. fluviatilis* and *L. planeri*. In two data sets (16S, COI), specimens of *L. fluviatilis* and *L. planeri* even had the same haplotype. A partition homogenecity test of all data sets did not predict significant incongruence between loci ($p > 0.03$). The phylogenetic analysis of the combined data set with 3790 aligned base pairs revealed two significantly supported clades. One included the members of the European species pair, but within this clade no significant separation between the two species was found (Fig. 3). The sister clade to the European species pair was in all analyses formed by *E. mariae*. Weighting according to the estimated TS/TV ratio and weighting of third codon positions in protein-coding genes had no effect on topologies (results not shown).

Phylogenetic analysis of the Lampetrini using Cyt *b*

The position of the European species pair and the Ukrainian brook lamprey within the Lampetrini was investigated using published sequences of Cyt *b* from the genera *Lethenteron*, *Lampetra*, *Entosphenus* (Supplemental Table S1[2]). Sequences of the genera *Petromyzon* and *Ichthyomyzon* were included as sister taxa. The topology of Cyt *b* reflected the genera of the Lampetrini (Fig. 4). Species considered to belong to *Entosphenus* (*Entosphenus similis*, *Entosphenus minimus*, *Entosphenus lethophagus*, *Entosphenus tridentatus*; Vladykov and Kott 1979*b*) formed a significantly supported clade. The European species pair *L. fluviatilis* – *L. planeri* and *Lampetra aegyptera* were clustered together with *E. mariae* (Fig. 4; 100%). Together with the monophyletic cluster

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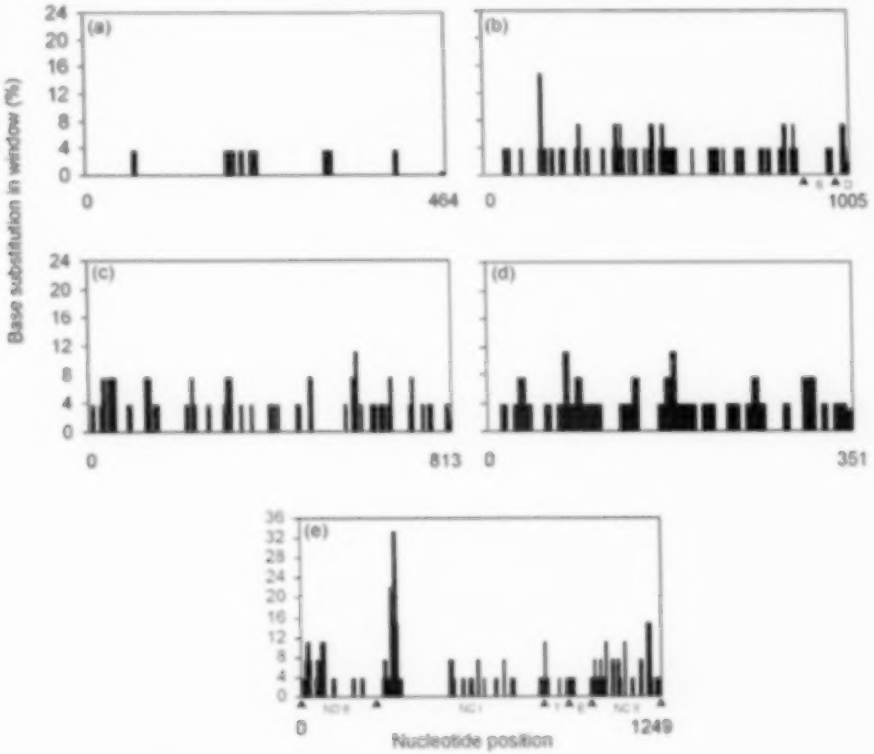
Fig. 2. Sliding-window analysis of (*a*) 16S ribosomal RNA, (*b*) cytochrome *c* oxidase subunit I (including tRNA-Ser (S) and tRNA-Asp (D)), (*c*) cytochrome *b*, (*d*) NADH dehydrogenase subunit 3, and (*e*) noncoding regions I (NC I) and II (NC II) (including partial NADH dehydrogenase subunit 6 (ND6), tRNA-Thr (T), and tRNA-Glu (E)). The percentage of variation within windows of nine bases was determined with three bases overlap, and the genetic variation was expressed as percentage of 27 possible base substitutions in one window of nine bases. Three classes of variation were defined: low-variable, 0%–20%; high-variable, 20%–40%; very high-variable, >40%.



of *Lampetra ayresii*, *Lampetra richardsoni*, and *Lampetra hubbsi*, they formed a weakly supported sister clade (50%) to the *Entosphenus* clade (Fig. 4). All other phylogenetic methods revealed *L. ayresii*, *L. richardsoni*, and *L. hubbsi* as one separate lineage (NJ, 98%; MP unweighted and weighted, 100%; ML, 98%; not shown), which was either placed as sister to *Entosphenus* (weighted MP, ML; not shown) or as equal sister group to all other lineages (unweighted MP, NJ; not shown). *Lethenteron reissneri*, *Lethenteron kessleri*, *Lethenteron appendix*, and *Lethenteron japonicum* were grouped in a *Lethenteron* lineage (Vladykov and Kott 1979*b*), which was found by all phylogenetic methods (BA, 50%; NJ, 93%; MP unweighted and weighted, 100%, ML, 82%; not shown).

Discussion

The species pair *Lampetra fluviatilis* and *Lampetra planeri*

The species status of members of lamprey species pairs has received considerable attention over the past 130 years. In 1883, Wajgel (Wajgel 1883) revised the available systematic of the European species pair *L. fluviatilis* and *L. planeri* and unified both species under the name *L. fluviatilis*. Owing to the absence of morphological differences in their ammocoetes, *L. planeri* was treated as the juvenile status of *L. fluviatilis*. The morphological similarity of the adults brought Enequist (1937) to treat them as ecological modifications of one species, whereas Weissenberg (1925) regarded them as clearly distinct biotypes. Others interpreted the different life modes to justify the retention of the species status for both species (Loman 1912). In the more recent literature, the European brook and river lampreys were kept as two separate but closely related species (Hardisty and Potter 1971; Holčík 1986).

Several recent studies addressed the problem of paired species using various genetic markers, but both mitochondrial as well as nuclear markers revealed a notorious lack of differentiation among closely related members of lamprey species pairs (e.g., Schreiber and Engelhorn 1998; Docker et al. 1999; Espanhol et al. 2007). The present study added several new mitochondrial loci to the already available data of protein-coding genes. The noncoding regions that are highly discriminatory in other taxa (e.g., Hoelzel et al. 1991; Lee et al. 1995) turned out to be more conserved than expected. No species-specific characters for both the European river lamprey and the brook lamprey in the 11 gene segments of the mitochondrial DNA were found.

The data support two alternative hypotheses: (*i*) *L. planeri* and *L. fluviatilis* belong to the same species and they should

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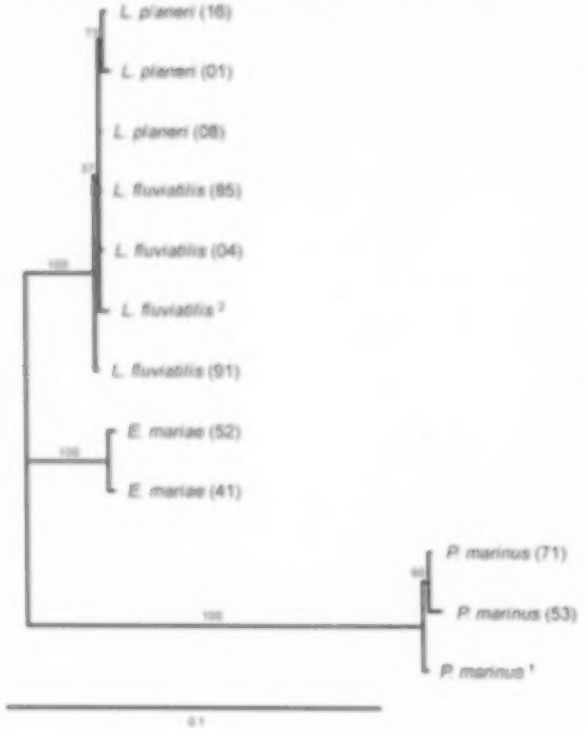
Fig. 3. Representative tree showing the results of the phylogenetic analyses among haplotypes (in parentheses) of lamprey species. This most probable tree is the result of the analysis of the combined data set based on the general time-reversible model with gamma (0.4806). All investigated mitochondrial loci (3790 bp) were included. Numbers above branches are likelihood values. Superscript numbers in the figure indicate the following references: [1], Lee and Kocher (1995; U11880); [2], Delarbre et al. (2000; Y18683).



be treated as "ecotypes" of the same species instead. The differences in the species' ecologies allow the occupation of a wide range of habitats and the usage of different food resources. The genetic distances between both species were, in all studied loci of our investigation, as high as within each species. This was also found in nuclear markers in the European species pair as well as in the American species pair (Beamish and Withler 1986; Engelhorn and Schreiber 1997; Krech 1997). A particular *L. planeri* population is more closely related to the respective sympatric *L. fluviatilis* population than to geographically distant conspecific populations, and both are members of a single Mendelian population (Krech 1997; Schreiber and Engelhorn 1998). Other genetic studies revealed nonmonophyly of the lamprey pair members as well (Espanhol et al. 2007; this study). All these results contradict a completed reproductive isolation but rather suggest ongoing gene flow and support the hypothesis of different ecotypes of a single species (e.g., Beamish and Withler 1986; Schreiber and Engelhorn 1998; Docker 2006). Furthermore, the possibility of multiple origins of brook lampreys from anadromous–parasitic river lampreys is supported by genetic data (Espanhol et al. 2007).

It is widely accepted that the brook lamprey has evolved from a polymorphic parasitic ancestor similar to the closely related extant parasitic species (e.g., Vladykov and Kott 1979*a*; Hardisty 1986*a*; Salewski 2003). Presumably every polymorphic lamprey population might give rise to one or several nonparasitic satellite forms (Beamish and Withler 1986; Hardisty 1986*a*; Beamish 1987). There is no evidence that a brook lamprey might have arisen from another brook lamprey (Vladykov and Kott 1979*a*). This phenomenon of the recurrence of species pairs is not only known in different lamprey genera worldwide, but also in fish genera like sticklebacks (*Gasterosteus*), lake whitefish (*Coregonus*), trout (*Salmo*), and Pacific salmon (*Oncorynchus*; reviewed in West-Eberhard 2003). Phenotypic plasticity, e.g., high variability in maturation, body size, and fecundity, seems to be the starting point for repeated evolution of phenotypic variants, like landlocked parasitic forms or brook lamprey forms (e.g., Hardisty 1986*a*; Goodwin et al. 2006). Natural selection acts on these novel phenotypes, and the fitness advantage of single variants, expressed, for example, as reproduction success due to assortative mating, might finally lead to reproductive isolation (West-Eberhard 2005). However, the stimulating environmental factors that initiate the production of phenotypic variants are still unknown. After Enequist (1937), there is a direct correlation between hydrogeographical characteristics of lakes and rivers and the evolution of brook lampreys. River lamprey larvae that drift passively into the sea develop into river lampreys. Otherwise, larvae that remain in river systems or that drift into lakes metamorphose into brook lampreys. The prevention of access to the sea during downstream migration through artificial barriers is also considered to trigger the metamorphosis to brook lamprey forms (Zanandrea 1961).

The alternative hypothesis (*ii*) is that *L. planeri* and *L. fluviatilis* are two closely related species that are reproductively isolated. The absence of genetic differentiation and the incomplete lineage sorting is the result of a recent postglacial separation event. The Pleistocene glaciations (2.4–0.01 million years ago BP) and associated climatic and hydrogeographic events were the factors that substantially affected the fauna and flora of North America and Europe and are considered to have triggered the speciation and distribution of the lampreys on the Northern Hemisphere (e.g., Enequist 1937; Zhukov 1965; Hardisty 1986*a*). The brook lamprey forms might have diverged from marine ancestors since the colonization of freshwater rivers and lakes after the retreat of the ice sheet. Genetic data support the possibility of multiple origins for the nonparasitic populations. This would explain the genetically closer relationship of sympatric populations of species pair members (Espanhol et al. 2007). The absence of genetic differences between brook and river lampreys contradicts a pre-Pleistocene divergence event.

In conclusion, our results clearly show that mtDNA is not able to discriminate the European brook (*L. planeri*) and river (*L. fluviatilis*) lampreys. This is in very close agreement with the results of a recently published study of Espanhol et al. (2007). Therfore, neither of the two hypotheses could be favoured. We would recommend, therefore, using other highly discriminatory nuclear genetic markers (e.g., microsatellites) in future investigation regarding the evolution of species pairs.

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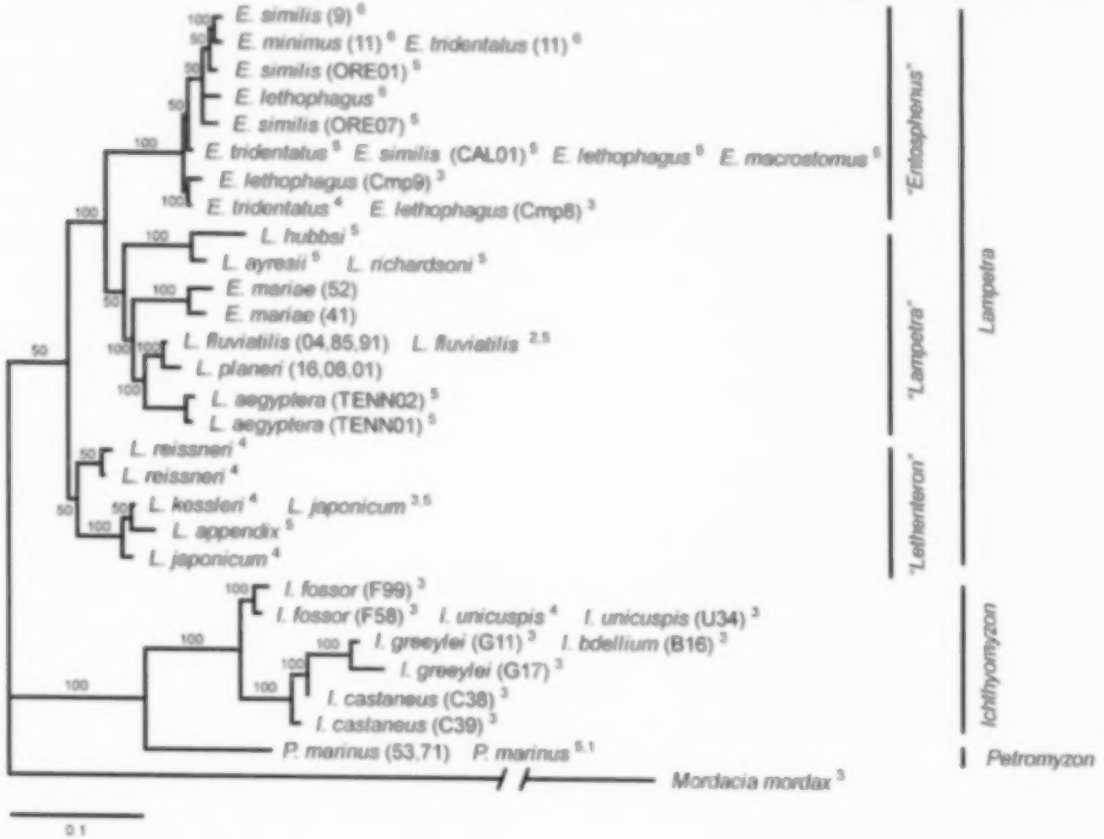
Fig. 4. Phylogenetic relationship of lamprey species based on the cytochrome *b* gene (Cyt *b*; 384 bp). *Mordacia mordax* was used as outgroup. The consensus phylogram from Bayesian analysis is based on the general time-reversible model with gamma (0.2566). Numbers above branches are posterior probability values. Data after species names in parentheses represent isolates. Superscript numbers in the figure indicate the following references: [1], Lee and Kocher (1995); [2], Delarbre et al. (2000); [3], Docker (2006); [4], Yamazaki et al. (2006); [5], Docker et al. (1999); [6], Lorion et al. (2000). For GenBank accession numbers of sequences, see Tables 1 and Supplemental Table S1.


E. similis (9) [6]
E. minimus (11) [6] E. tridentatus (11) [6]
E. similis (ORE01) [5]
E. lethophagus [6]
E. similis (ORE07) [5]
E. tridentatus [5] E. similis (CAL01) [5] E. lethophagus [5] E. macrostomus [6]
E. lethophagus (Cmp9) [3]
E. tridentatus [4] E. lethophagus (Cmp8) [3]
L. hubbsi [5]
L. ayresii [5] L. richardsoni [5]
E. mariae (52)
E. mariae (41)
L. fluviatilis (04,85,91) L. fluviatilis [2,5]
L. planeri (16,08,01)
L. aegyptera (TENN02) [5]
L. aegyptera (TENN01) [5]
L. reissneri [4]
L. reissneri [4]
L. kessleri [4] L. japonicum [3,5]
L. appendix [5]
L. japonicum [4]
I. fossor (F99) [3]
I. fossor (F58) [3] I. unicuspis [4] I. unicuspis (U34) [3]
I. greeleyi (G11) [3] I. bdellium (B16) [3]
I. greeleyi (G17) [3]
I. castaneus (C38) [3]
I. castaneus (C39) [3]
P. marinus (53,71) P. marinus [5,1]
Mordacia mordax [3]
"Entosphenus"
"Lampetra"
"Lethenteron"
Lampetra
Ichthyomyzon
Petromyzon
0.1

The phylogenetic position of *Eudontomyzon mariae*

Since its description, the phylogenetic status of the genus *Eudontomyzon* was often discussed. This Palaearctic genus comprises currently four species: three of them, *E. mariae*, *Eudontomyzon hellenicus*, and *Eudontomyzon stankokaramani*, have a nonparasitic mode of life, whereas *Eudontomyzon danfordi* is an anadromous, parasitic species (Holčík 1986; Holčík and Sorić 2004). There are indications of another undescribed parasitic species (Holčík 1986; Holčík and Renaud 1986; Renaud 1997).

In the past, *Eudontomyzon* was considered to be a subgenus of either *Petromyzon* (Creaser and Hubbs 1922) or *Lampetra* (Berg 1931; Bailey 1980; Potter 1980). The unique dentition found in all species of *Eudontomyzon* seemed to justify this opinion (Holčík 1986).

The extended phylogenetic analyses in the present study using 384 bp of the Cyt *b* gene revealed a close relationship of *E. mariae* to *L. fluviatilis* – *L. planeri* and to *L. aegyptera*. This is in accordance with the morphological studies of Gill et al. (2003), which indicated a sister group relationship of *E. morii* and *E. danfordi* to *Lampetra*. But some attention should be given to the fact that several morphological characters in *Eudontomyzon* show extreme variability or at least clinal variation (Holčík and Delić 2000).

The present day discontinuous distribution of the representatives of the genus *Eudontomyzon* is remarkable, and there are two possible explanations: either the genus is of monophyletic origin as proposed by morphology and the present day distributions are the relicts of a former continous distribution, or the species of the genus arose independently from different parasitic forms. Zhukov (1965, 1968) assumed an extinct anadromous Black Sea lamprey as ancestor of *E. mariae* and the European species pair. This would be consistent with the results of our molecular results. The date of separation of *L. fluviatilis* and *E. mariae* inferred from the genetic distances should be earlier than that of *L. fluviatilis* and *L. planeri*, which must be dated most likely postglacial. We propose that *E. mariae*, which was deemed to be identical with *L. planeri* in the past (*Lampetra mariae*; Berg 1931), should be placed back in the genus *Lampetra*. The discriminating morphological characters between both species are the villiform teeth and greater length of adults

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and ammocoetes in *E. mariae* (up to 222 mm vs. up to 166 mm total length in adults and up to 230 mm vs. 180 mm total length in ammocoetes; Vladykov and Follett 1967; Holčík 1986; Holčík and Renaud 1986). Both species were described to occur sympatrically in the upper tributaries of the rivers Neman and Vistula (Baltic Sea drainage system), the Danube River, and the upper and middle part of the Volga river basin (Zhukov 1965; Kappus et al. 1995; Levin and Holčík 2006). Hybrids between *L. planeri* and *E. mariae* with intermediate characters were described by Rembiszewski (1968) from the river Jeziorka (Poland). This underlines their close relationship. In allozyme electrophoresis, both species were clearly distinct in five markers and no hybrid individuals could be identified among individuals from the upper Danube River (Engelhorn and Schreiber 1997).

Two other nonparasitic species whose position and status are not assured yet (Zanandrea 1961; Hubbs and Potter 1971; Potter 1980) also revealed a closer relationship to *L. fluviatilis*: *Lampetra aegyptera* and *Lethenteron zanandreai*. *Lampetra aegyptera* was regarded as a derivative of the parasitic *L. ayresii* (Vladykov and Kott 1979*a*), but the results of the genetic analyses (Docker et al. 1999; present study) showed a closer relationship to the European species pair *L. fluviatilis* – *L. planeri*. An ancestor similar to *L. fluviatilis* as deduced by Docker et al. (1999) is most likely. The same may be the case for *L. zanandreai*, and a reintegration of this species in the genus *Lampetra* is advisable (Vladykov 1955; Docker et al. 1999). The monophyly of the *L. fluviatilis*-type ancestor lineage comprising the species *L. fluviatilis*, *L. planeri*, *E. mariae*, and *L. aegyptera* is well supported in all phylogenetic reconstructions irrespective of the analysed gene segment (Docker et al. 1999; Yamazaki et al. 2006; present study).

Summarizing, the Ukrainian brook lamprey, *E. mariae*, should be reincluded in the genus *Lampetra*. By adding genetic data from the other species belonging to *Eudontomyzon*, it will be possible to clarify the generic status (monophyly vs. polyphyly) of *Eudontomyzon* and its relationship to the other genera of the Lampetrini. All genetic studies so far are hampered by the fact that not all known lamprey species are included. Different analyses shed light on particular questions of the status of extant lamprey genera and species and the lamprey phylogeny. Future research should therefore strive to comprise all known species to clarify the unresolved questions of lamprey phylogeny.

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Trophic studies in a high-latitude fjord ecosystem — a comparison of stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) and trophic-level estimates from a mass-balance model

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Abstract: Stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ were used to examine food sources and trophic structure of 65 taxa, representing 19 ecological groups, in a high-latitude ecosystem. Discrimination was made between pelagic and benthic carbon sources, where feeding in most cases reflected the habitat. Trophic levels from these analyses, TL_N, were compared with corresponding levels estimated by an Ecopath mass-balance model, TL_E, constructed independently of the isotope data. The good correlation between the two methods ($r^2 = 0.72$) supports the diet composition and the grouping of taxa into ecological groups in the model. However, when estimates diverged, this was often explained by the analyses of few taxa, taxa that were not the most representative for the group, or the analyses of specimens from a limited size range. Some assumed detrivores were assigned high TL_N in favour of an abundant microbial community in the sediments. High TL_N estimates for many invertebrate taxa, combined with relatively low TL_N for fishes, suggest that parts of the benthic food web are decoupled from the classical food web.

Résumé : Les isotopes stables de $\delta^{13}C$ et de $\delta^{15}N$ nous ont servi à examiner les sources alimentaires et la structure trophique chez 65 taxons, représentant 19 groupes écologiques, dans un écosystème de haute latitude. Il a été possible de discriminer entre les sources pélagiques et benthiques de carbone et l'alimentation reflète l'habitat, dans la plupart des cas. Nous avons comparé les niveaux trophiques obtenus dans ces analyses, TL_N, aux niveaux correspondants, TL_E, estimés par le modèle de bilan de masse d'Ecopath construit indépendamment des données isotopiques. La forte corrélation entre les deux méthodes ($r^2 = 0,72$) confirme la composition des régimes alimentaires et le regroupement des taxons en groupes écologiques dans le modèle. Cependant, les cas de divergence entre les estimés s'expliquent souvent par l'analyse d'un petit nombre de taxons, celle de taxons qui ne sont pas les plus représentatifs du groupe ou celle de spécimens d'une gamme restreinte de tailles. Certains détritivores présumés ont obtenu une valeur élevée de TL_N, ce qui laisse croire à une abondante communauté microbienne dans les sédiments. Les valeurs élevées de TL_N estimées chez plusieurs invertébrés, combinées aux valeurs relativement basses de TL_N chez les poissons semblent indiquer que certaines parties du réseau alimentaire sont découplées par rapport aux réseaux alimentaires classiques.

[Traduit par la Rédaction]

Introduction

The investigation of trophic linkages is a fundamental requirement for understanding the energy flows from primary producers to higher level consumers in marine food webs. Since the introduction of the food chain concept by Elton (1927), the understanding of trophic levels has undergone a conceptual change from trophic levels articulated as integers to food webs where organisms derive food from different parts of the web, resulting in fractional trophic levels (Odum and Heald 1972).

In addition to direct observations and gut analyses, a number of chemical tracing techniques have been used to elucidate the food sources of marine animals. These include analyses of fatty acid composition of animals and their potential food sources (Falk-Petersen et al. 2000), following radioisotope labels through food webs (Marples 1966), and analysing the stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ (Hobson and Welch 1992; Fisk et al. 2003; Tamelander et al. 2006). Two widely used methods for estimating trophic levels are the $\delta^{15}N$ stable isotope method (Hobson and Welch 1992; Fisk et al. 2003; Tamelander et al. 2006) and mass-balance models (Christensen 1995; Harvey et al. 2003; Pedersen et al. 2008). Few attempts have been made, however, to independently compare and validate these methods (Kline and Pauly 1998; Mathisen and Sands 1999; Dame and Christian 2008). This procedure is important because it creates a valuable feedback for model construction, analyses, and validation.

Several fjord ecosystems have been subjected to quantitative studies and ecosystem modelling (Aksnes and Lie 1990; Bax and Eliassen 1990; Salvanes et al. 1992). For Sørfjord,

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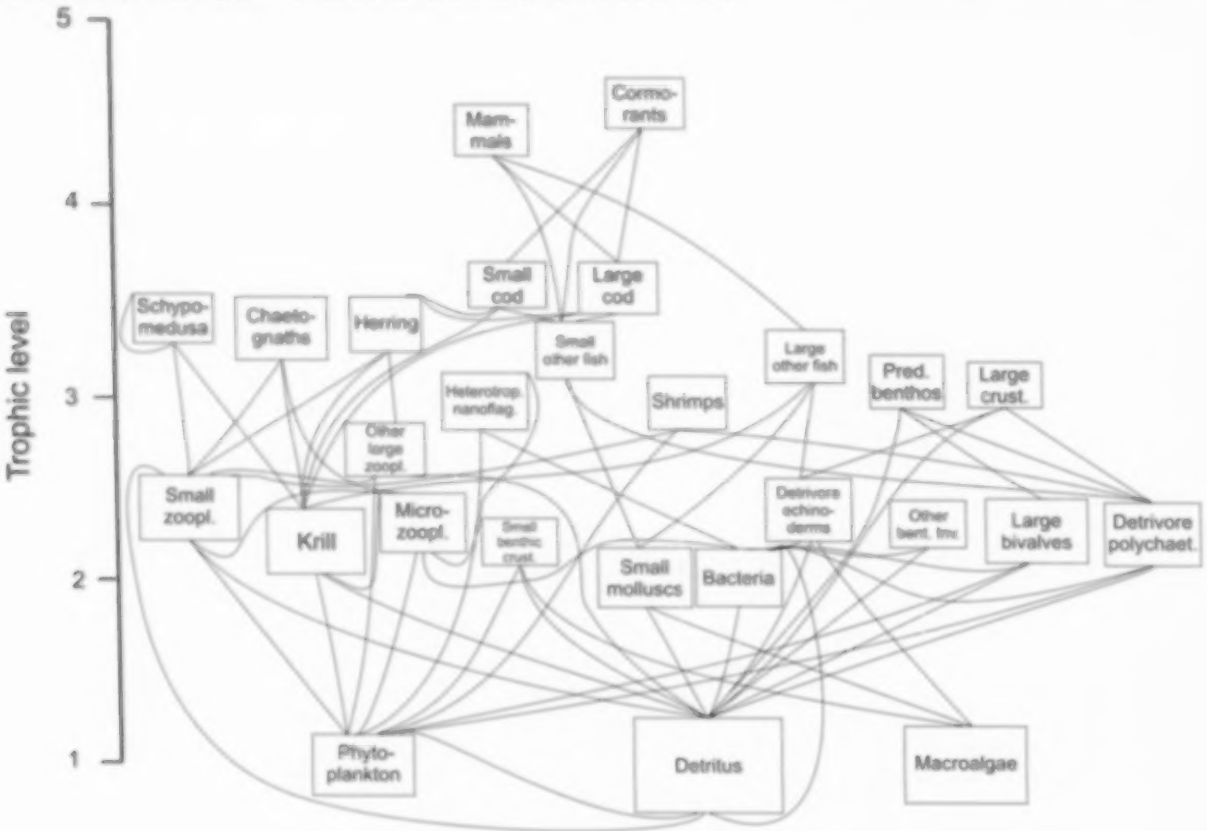
Fig. 1. Overview of the Sørfjord food web as assessed by an Ecopath mass-balance model (Pedersen et al. 2008). For each ecological compartment, the lines entering the bottom of the box show the three most important diet links. Lines are leaving from the top of the prey boxes. The three most important sources for the detritus compartment are also shown.



North Norway, information from a number of single quantitative studies was synthesized in a mass-balance model, using Ecopath, by Pedersen et al. (2008) (Fig. 1). With supplementary data from other fjords and coastal areas, the carbon flow through 27 ecological groups was balanced from information on biomass, production–biomass ratios, mortalities, consumption, catches, and dietary composition. The Sørfjord ecosystem during 1993–1996 (the model period) was only lightly exploited, with a fish community dominated by Atlantic cod (*Gadus morhua*), and with a high biomass of pelagic and benthic invertebrates (Pedersen and Pope 2003; Zhou et al. 2005; Nilsen et al. 2006). The Ecopath model reveals a food web with mammals, cormorants, and cod as the major top predators. Cod is the main target of the limited small-scale fishery, and trawling is prohibited. Phytoplankton, macroalgae, and detritus are the major food sources for lower trophic-level invertebrates, while krill is the major food source for the fish groups (Fig. 1). Although the food web components resemble those in open systems, the physical conditions in this semi-enclosed fjord system are more stable, and the limited exchange of organisms with surrounding areas is largely controlled by advective processes (tidal currents and estuarine circulation). The abiotic and biotic conditions in Sørfjord support a rich and relatively undisturbed fauna, which makes it a good location for studying trophic linkages.

An important prerequisite for the building of functional food web models is reliable dietary information to assess where the energy originates from and how it flows. For invertebrate taxa, in particular, there are large challenges related to this, mainly as a result of the small size of the individuals. Also, dietary analyses based on gut analyses can only provide measurements of recently ingested food and are confined to the possibility of identifying the food item. The naturally occurring stable carbon (^{13}C) and nitrogen (^{15}N) isotopes provide time-integrated averages of assimilated food and therefore add valuable information to conventional dietary analyses (Hopkins et al. 1989). The two isotopes are differentially enriched over trophic levels, with enrichment factors of 0.6‰–1‰ and 3‰–4‰ per trophic level for $\delta^{13}C$ or $\delta^{15}N$, respectively (Hobson and Welch 1992; Post 2002; Søreide et al. 2006*a*). In fjords, carbon mainly originates from phytoplankton, macroalgae, benthic microalgae, or allochthonous plant material. Provided that the different carbon sources have distinct $\delta^{13}C$ signatures, $\delta^{13}C$ can be used to discriminate among these and to assess their importance for the various consumers. The higher enrichment factor of $\delta^{15}N$ makes this more suitable for the es-

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timation of the trophic levels of the consumers in the ecosystem (Hobson and Welch 1992; Post 2002).

Many marine food webs have experienced large changes in the structure and flow patterns following changes or perturbations in the biotic and abiotic environment (Frank et al. 2005). These are often attributed to changes in species composition, but ecological niches and trophic positions are also affected (Gorokhova et al. 2005). A better understanding of these aspects and of the stabilizing or destabilizing forces of omnivorous connections (Vandermeer 2006) is necessary for the future management of marine ecosystems. The main objectives have been to use stable isotopes to validate and contribute to the diet information database of some selected species in an ecosystem that has previously been modelled by an Ecopath mass-balance model and evaluate the composition of the ecological groups that were defined in this model (Pedersen et al. 2008). This approach is useful for the assessment of model structure, both in Sørfjord and in other ecosystems, and generates valuable information for the building of new food web models.

Materials and methods

Study area

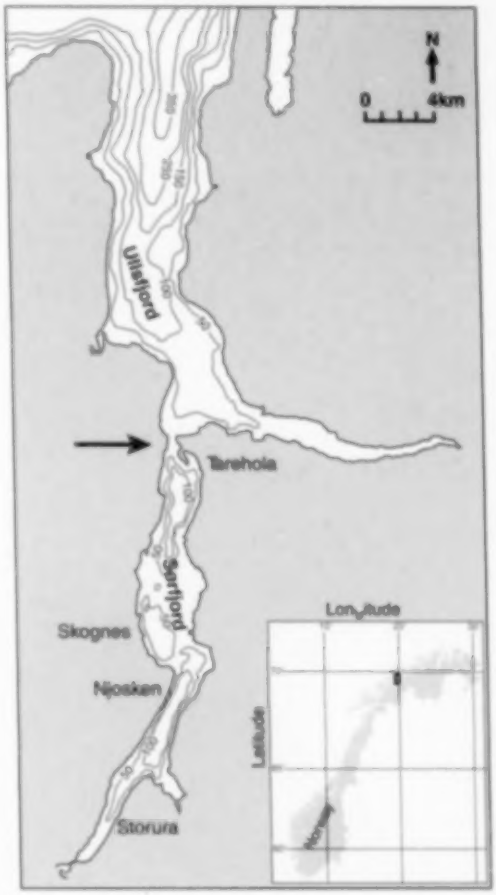
Sørfjord is a high-latitude fjord located at 69°40′N, 19°40′E, North Norway (Fig. 2). It is 27 km long and 3 km wide. A narrow and shallow sill, 300 m wide and 8 m deep, separates it from Ullsfjord (Fig. 2). The bathymetry features two deep basins (Tarehola and Njosken), both ~130 m deep, separated by a shallow (Skognes) of 50 m. A second shallow of 50 m (Storura) is located at the head of the fjord (Fig. 2). Tidal exchange with warm and saline Atlantic water flowing in from Ullsfjord creates a turbulent and well-mixed water column in Tarehola throughout the year. The inner subareas (Skognes, Njosken, and Storura) are colder and less saline, and a thermocline develops at approximately 50 m depth during summer. Water temperature may reach 9–13 °C in the surface water during summer, but is normally <3 °C in the bottom water throughout the year.

Sample materials

Attempts were made to sample species or taxa representative for the majority of the ecological groups defined in the mass-balance model (Ecopath model) constructed for Sørfjord (Pedersen et al. 2008). The selection of food web components for the stable isotope analyses was primarily based on samples collected from the different subareas of Sørfjord during January 2006. However, krill was absent in the samples from 2006 and was collected in February 2007. Sediments and some supplementary benthic invertebrates sampled in January 2005 were also included to obtain more replicates.

The different bathymetric and hydrographical conditions described earlier can potentially result in local differences in food availability, but for economical reasons, separate analyses between subareas (Tarehola and Njosken) were made only for the dominating fish species: Atlantic cod (*Gadus morhua*) and long rough dab (American plaice, *Hippoglossoides platessoides*) (Table 1, Fig. 2). For the remaining species, the replicate samples were collected from

Fig. 2. Sørfjord, North Norway, with its four subareas indicated. The arrow shows the location of the sill at the fjord entrance.



different subareas when possible to give a more representative fjord average.

Fish were collected by both bottom and pelagic trawls. The bottom trawl used to catch demersal species was a Campelen shrimp trawl (1300 meshes circumference) equipped with a rockhopper gear and a 20 mm mesh size inner net-mounted in the cod end. Pelagic fishes were sampled with a Harstad trawl with a 10 mm inner net in the cod end. Both trawls were towed at 2 kn (1 kn = 1.852 km·h^{-1}) for 20–30 min. Most benthic invertebrates were sampled by triangular dredge, but additional invertebrates and macroalgae were also collected from the bottom trawl hauls. Benthic infaunal invertebrates and sediments were sampled with a van Veen grab, and a WP2 net and pelagic trawl were used to sample pelagic invertebrates.

With the exception of polychaetes, all pelagic and benthic invertebrates were kept alive in filtered seawater for ~24 h to clear the guts (Hobson and Welch 1992; Tamelander et al. 2006) before freezing. Fish were identified and lengths measured prior to freezing. For most fish, only small pieces of muscle tissue were frozen, but the small *Benthosema glaciale* was frozen whole.

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Table 1. Stable isotope values (‰) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) for all analysed taxa and ecological groups (given as mean ± SD), number of samples analysed for stable isotopes (n), trophic levels estimated from stable isotopes (TL_N) and an Ecopath model (TL_E, Pedersen et al. 2008), the biomass contribution of each taxon to their respective ecological group (contribution, %), and percentage dietary importance of benthic carbon for the different ecological groups (benthic ^{13}C, %).

Main group – ecological group	Taxon	$\delta^{13}C$	$\delta^{15}N$	n	TL_N	TL_E	Contribution (%)	Benthic ^{13}C (%)
Fish								
Large cod (LCO)		**–18.2**	**15.3**	**10**	**3.9**	**3.6**		**74**
	Gadus morhua (Njosken, 35–70 cm)	–19.2±0.1	15.0±0.7	3	3.8		33.3	
	Gadus morhua (Tarehola, >70 cm)	–17.5±0.1	16.1±1.1	4	4.2		33.3	
	Gadus morhua (Tarehola, 35–70 cm)	–18.2±0.1	14.6±0.2	3	3.7		33.3	
Small cod (SCO)		**–18.6**	**14.3**	**4**	**3.6**	**3.6**		**68**
	Gadus morhua (Tarehola, <35 cm[a])	–18.6±0.3	14.3±0.6	4	3.6		100	
Large other fish (LOF)		**–17.7**	**13.9**	**22**	**3.5**	**3.2**		**88**
	Anarhichas lupus	–17.3±0.3	13.0±0.6	3	3.2		32.0	
	Hippoglossoides platessoides (Tarehola, >35 cm)	–18.0±0.2	14.2±0.7	3	3.6		15.0	
	Melanogrammus aeglefinus	–17.8±0.2	14.8±0.1	4	3.8		37.0	
	Microstomus kitt	–18.5±0.7	13.6±0.2	3	3.4		6.0	
	Pleuronectes platessa	–17.7±0.9	12.7±1.4	5	3.1		7.0	
	Raja radiata	–17.3±1.1	13.9±0.5	4	3.5		3.0	
Small other fish (SOF)		**–18.9**	**14.6**	**17**	**3.7**	**3.3**		**61**
	Benthosema glaciale	–22.4±1.1	11.3±0.4	4	2.7		<0.1	
	Hippoglossoides platessoides (Tarehola, <35 cm)	–19.1±0.1	14.7±0.8	3	3.7		93.4	
	Hippoglossoides platessoides (Njosken, <35 cm[b])	–18.4±0.8	14.0±0.8	4	3.5		<0.1	
	Lumpeninae	–21.9±0.4	12.3±0.6	3	3.0		6.5	
	Mallotus villotus	–20.5±0.2	14.3±0.1	3	3.6			
Herring (HER)		**–21.3**	**12.0**	**4**	**2.9**	**3.4**		**26**
	Clupea harengus	–21.3±0.3	12.0±0.5	4	2.9		100	
Pelagic invertebrates								
Krill (KRI)		**–22.4±0.5**	**11.1±0.6**	**6**	**2.6**	**2.2**		**8**
	Meganyctiphanes norvegica	–22.8±0.5	11.2±0.2	3	2.6		50.0	
	Thysanoessa raschii	–22.0±0.5	11.0±0.9	3	2.5		50.0	
Small zooplankton (SZO)		**–24.1**	**9.1**	**3**	**2.0**	**2.4**		**0**
	Calanus finmarchicus	–24.1±0.1	9.1±0.1	3[d]	2.0		100	
Other large zooplankton (OLZ)		**–22.8**	**14.0**	**4**	**3.4**	**2.7**		**0**
	Hyperia galba[c]	–22.8±0.1	14.0±0.7	4[e]	3.4		100	
Benthic invertebrates								
Shrimps (SHR)		**–17.5**	**13.0**	**7**	**3.1**	**3.0**		**98**
	Pandalus borealis	–17.5±0.8	13.0±0.9	4	3.1		90.0	
	Sabinea septemcarinata	–17.0±0.2	12.9±0.2	3	3.1		10.0	
Large crustaceans (LCR)		**–20.0**	**9.1**	**15**	**2.0**	**3.0**		**66**
	Hyas araneus[c]	–20.6±0.8	8.7±1.0	4	1.9		56.0	
	Hyas coarctatus[c]	–20.9±0.3	9.3±0.9	4	2.0		14.0	
	Pagurus bernhardus	–18.6±1.4	9.5±1.1	4	2.1		15.0	
	Pagurus pubescens	–18.4±0.2	9.6±0.4	3	2.1		15.0	

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Table 1 (*continued*).

Main group – ecological group	Taxon	$\delta^{13}C$	$\delta^{15}N$	*n*	TL_N	TL_C	Contribution (%)	Benthic ^{13}C (%)
Predatory benthos (PRB)		**–17.9**	**11.6**	**25**	**2.7**	**3.0**		**97**
	Asterias rubens[c]	–17.6±0.6	10.1±0.3	3	2.3		20.0	
	Buccinum sp.	–16.8±0.6	11.7±0.9	4	2.8		30.0	
	Eunice norvegica	–20.7±1.1	10.0±0.3	3	2.2		8.4	
	Lumbrineris fragilis	–19.1±0.6	12.9±0.3	4	3.2		2.0	
	Nephtys sp.	–18.8±0.7	12.9±0.6	4	3.1		29.2	
	Neptunea sp.	–17.1±0.6	11.6±0.8	3	2.7		10.0	
	Polynoidae indeterminate	–19.6±0.1	10.1±1.0	4	2.3		0.4	
Detrivore polychaetes (DPO)		**–20.1**	**11.5**	**6**	**2.7**	**2.3**		**53**
	Maldanidae indeterminate	–19.5±0.5	13.1±1.2	3	3.2		65.0	
	Myriochele oculata[c]	–21.2±0.5	8.4±0.6	3[f]	1.8		35.0	
Small benthic crustaceans (SBC)		**–20.3**	**5.7**	**3**	**1.0**	**2.2**		**76**
	Gammarus sp.[c]	–20.3±0.5	5.7±0.9	3	1.0		100	
Small molluscs (SMO)		**–16.9**	**9.9**	**12**	**2.2**	**2.0**		**100**
	Gibbula cineraria	–16.4±0.8	9.3±0.7	4	2.0		31.5	
	Lacuna divaricata	–17.0±1.2	7.4±0.3	4[f]	1.5		31.5	
	Lepidochitona cinerea	–17.3±1.0	12.6±0.8	4	3.0		37.0	
Large bivalves (LBI)		**–18.9**	**9.0**	**18**	**2.0**	**2.2**		**88**
	Arctica islandica	–18.8±0.3	9.0±0.4	4	2.0		83.0	
	Chlamys islandica	–19.9±0.2	8.9±0.4	3	1.9		3.5	
	Modiolus modiolus	–19.7±0.5	9.4±0.3	4	2.1		3.5	
	Musculus niger	–18.8±0.4	8.5±0.6	3	1.8		8.0	
	Pseudamussium septemradiatum	–19.2±0.4	12.4±0.3	4	3.0		2.0	
Detrivore echinoderms (DEC)		**–17.0**	**13.0**	**19**	**3.1**	**2.4**		**100**
	Ctenodiscus crispatus	–16.2±0.6	14.6±1.4	4	3.6		69.0	
	Cucumaria frondosa	–17.2±0.2	10.9±0.1	3	2.5		10.0	
	Ophiopholis aculeata[c]	–20.2±0.3	10.0±0.5	3	2.3		5.5	
	Ophiura sarsi[c]	–18.0±1.2	11.1±0.5	6	2.6		5.5	
	Strongylocentrotus droebachiensis	–19.3±0.1	7.0±0.4	3	1.4		10.0	
Other benthic invertebrates (OBI)		**–20.5**	**9.4**	**6**	**2.1**	**2.3**		**56**
	Hemithiris psittacea	–20.7±0.4	8.7±0.2	3	1.9		78.0	
	Pycnogonum littorale	–20.0±0.4	11.6±0.5	3	2.7		22.0	
Macroalgae–sediment								
Macroalgae (MAC)		**–16.4**	**4.5**	**52**	**0.6**	**1.0**		
	Alaria esculenta	–22.8±3.3	6.5±1.1	8	1.2		0.8	
	Ascophyllum nodosum	–15.6±0.1	4.2±0.4	5	0.6		78.0	
	Desmarestia aculeata	–24.8±2.6	7.0±1.0	5	1.4		0.8	
	Fucus serratus	–17.6±1.4	5.0±0.9	2	0.8		11.0	
	Fucus vesiculosus	–17.3±1.8	4.6±1.1	9	0.7		5.0	
	Laminaria digitata	–20.9±1.6	4.9±0.4	4	0.8		0.8	
	Saccharina latissima	–20.7±2.4	5.4±0.6	12	0.9		0.8	
	Phycodrys rubens	–34.5±0.7	7.3±0.2	2	1.4		0.8	

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Table 1 (*concluded*).

Main group – ecological group	Taxon	$\delta^{13}C$	$\delta^{15}N$	*n*	TL_N	TL_E	Contribution (%)	Benthic ^{13}C (%)
	Polysiphonia lanosa	–22.8±0.5	5.9±0.4	3	1.1		0.8	
	Ptilota serrata	–34.0	6.4	1	1.2		0.8	
	Ulva intestinalis	–18.6	6.3	1	1.2		0.8	
Sediment (SED)		**–21.3**	**5.4**	**11**	**0.9**	**1.0**		
	<50 m[e]	–21.4±0.3	5.6±0.6	5	1.0		33.3	
	Tarehola, >50 m[e]	–21.1±0.5	5.0±0.2	3	0.8		33.3	
	Skognes, Njosken, Storura: >50[e]	–21.3±0.4	5.3±0.7	3	0.9		33.3	

Note: Sixty-five taxa are merged into 19 ecological groups (bold) and four main groups, where ecological groups correspond with groups used in the Sørfjord Ecopath model (Pedersen et al. 2008). Abbreviations for ecological groups, used in figures and tables, are given in parentheses. The number of analysed samples (*n*) is the number of replicate samples analysed for each taxon or group. In most cases, replicate samples refer to one individual organism, or part of one organism, and were collected from different subareas. However, for small species, several individuals were pooled into one replicate (see footnotes *d*, *e*, and *f*), and analyses of the two dominating fish species, *Gadus morhua* and *Hippoglossoides platessoides*, were made separately for subareas (Tarehola and Njosken) and size groups (<35, 35–70, and >70 cm, where 35 cm also corresponds to the length separating small and large other fish in the Ecopath model). Sediments resemble detritus in the Ecopath model and were also analysed separately for subareas and depths. Average values given for ecological groups were estimated from the percentage contribution of biomass each taxon had to the ecological group, accounting only for the analysed taxa and not for other taxa included in the corresponding Ecopath group. Percentage ^{13}C of benthic origin was estimated by a mixing equation (McConnaughey and McRoy 1979) using values back-calculated from *Calanus finmarchicus* and cod diet as representative for pelagic and benthic carbon sources, respectively.

[a]Includes one individual of 37 cm.

[b]Includes one individual of 36.6 cm.

[c]Treated with HCl.

[d]20 individuals pooled into one replicate.

[e]3 individuals pooled into one replicate.

[f]5 individuals pooled into one replicate.

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Sample preparation

Macroalgae, fish, invertebrates, and sediments were dried at 60 °C in a drying cabinet for 48 h and subsequently pulverized using a glass mortar and pestle. All animal taxa and sediments were first rinsed in distilled water to remove salts. Only a small piece of muscle was prepared for most fish, but *B. glaciale* was prepared intact. Soft tissue was removed and prepared for invertebrate taxa when possible. Many invertebrates have carbonate-rich structures that can hamper isotope results and were therefore dried, soaked in 1 mol·L^{-1} HCl for 24 h to remove carbonates, cleaned with distilled water, and dried again before they were pulverized (Table 1). Acid treatment was also performed for the sediment samples. Dried and pulverized samples were stored in Eppendorf tubes pending analyses.

Stable isotope analyses

Sixty-five taxa, distributed over 244 replicates, were analysed. These included the separation between subareas (Tarchola and Njosken) and length groups for cod (<37, 37–70, >70 cm) and long rough dab (<36.6, >36.6 cm) (Table 1). A length of 35 cm was used to separate between the length groups in the Ecopath model because of the selectivity of the trawl. Although attempts were made to use the same separation here, an individual specimen of 37 and 36.6 cm was included in the small cod and small other fish groups, respectively, because of failure to sample enough small specimens. Sediments from different subareas and depths were also analysed separately (Table 1). Stable isotope analysis was performed on at least three replicates from each taxon. The replicates represented single individuals or part of one individual depending on the size. However, for some of the smallest invertebrate species, 3–20 individuals were pooled into one replicate to ensure sufficient material for analyses (Table 1). For some of the macroalgae, different parts of the plants (e.g., stipes, lamina) were treated as replicates.

Stable carbon and nitrogen isotope analyses were performed at the Institute for Energy Technology in Kjeller, Norway, during November 2006 (krill were analysed in April 2007). Approximately 1 mg of the dried sample material was transferred to 9 mm × 15 mm tin capsules. Samples were combusted in the presence of O_2 and Cr_2O_3 at 1700 °C using a Carlo Erba NCS 2500 element analyser coupled to a Micromass Optima IRMS.

Stable isotopes are expressed in δ notation as the deviation from international standards in parts per thousand (‰) according to the following equation:

$$(1) \qquad \delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where X is ^{13}C or ^{15}N, and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Standards for $\delta^{13}C$ and $\delta^{15}N$ were calibrated against IAEA-N-1, IAEA-N-2, and USGS-24.

Carbon sources

Discrimination amongst all the potential carbon sources (phytoplankton, macroalgae, benthic microalgae, and allochthonous plant material) could not be made since samples were collected during winter when phytoplankton was absent and rivers were frozen, in addition to the analyses of sediments as a single batch. It was therefore attempted to identify and separate between an average for pelagic-derived and benthic-derived carbon. A $\delta^{13}C$ value believed to be representative of pelagic carbon represented by phytoplankton was back-calculated from the $\delta^{13}C$ value of the copepod *Calanus finmarchicus*. Since this copepod mainly feeds on phytoplankton (Hopkins et al. 1989), the $\delta^{13}C$ value representing the latter was calculated under the assumptions that their respective trophic levels are 2.0 and 1.0 and that ^{13}C is enriched by 0.8‰ per trophic level (Fisk et al. 2003; Søreide et al. 2006*a*).

Benthic carbon sources could potentially be macroalgae, benthic microalgae, or materials sedimenting out from the pelagic zone reaching the benthos as detritus. To give an average for the benthic carbon in this system, four alternatives were considered representative based on the samples available: (*i*) the average macroalgae value, (*ii*) the weighted macroalgae average value, accounting for the relative contribution to biomass of the different species that were analysed, (*iii*) the average sediment value, and (*iv*) an integrated value back-calculated from the proportion of invertebrate benthic organisms in the diet of large cod used in the Ecopath model (Pedersen et al. 2008). The latter value was estimated under the assumptions that the TL_N (denotes trophic level estimated from $\delta^{15}N$) of large cod was one trophic level higher than its prey, accounting for the relative contribution (to biomass) of different benthic prey in the cod diet, and a ^{13}C enrichment by 0.8‰ per trophic level.

The percentage importance of benthic derived carbon in the diet of the ecological groups was calculated from the mixing equation of McConnaughey and McRoy (1979):

$$(2) \qquad \%C_{benthic\text{-}derived} = [(\delta^{13}C_{consumer} - \delta^{13}C_{phytoplankton} - I) / (\delta^{13}C_{benthic\ carbon} - \delta^{13}C_{phytoplankton})] \times 100$$

where I is $0.8TL_{N\,consumer}$ following the 0.8‰ enrichment per trophic level, $\delta^{13}C_{consumer}$ is the value of the consumer of interest, and $\delta^{13}C_{phytoplankton}$ and $\delta^{13}C_{benthic\ carbon}$ are the pelagic and benthic carbon averages, respectively. With only two major carbon sources identified, the corresponding importance of pelagic carbon is $100\% - (\%C_{benthic\text{-}derived})$.

Trophic levels estimated from $\delta^{15}N$ values (TL_N)

TL_N estimates were based on the ^{15}N enrichment per trophic level ($\Delta\delta^{15}N$) according to the formula

$$(3) \qquad TL_N = (\delta^{15}N_{consumer} - \delta^{15}N_{base})/\Delta\delta^{15}N + TL_{base}$$

$\delta^{15}N_{consumer}$ is the $\delta^{15}N$ of the taxa in question, while $\delta^{15}N_{base}$ and TL_{base} are the average $\delta^{15}N$ and trophic level of the baseline, respectively (see Søreide et al. 2006*a*). *Calanus finmarchicus* was selected as the baseline consumer, with a TL_N of 2.0 based on the assumption that it is a phytoplankton feeder. For invertebrates, macroalgae, and sediments, a $\Delta\delta^{15}N$ of 3.4‰ was applied. This factor is adopted from Søreide et al. (2006*a*) and is based on analyses of samples from the Barents Sea, a high-latitude area adjacent to Sørfjord. A separate $\Delta\delta^{15}N$ was selected for fish, depending on whether whole fish (2.9‰) or fish muscle (3.2‰) was analysed (Sweeting et al. 2007). The different enrichment factors indirectly account for variations in enrichment related to season, type of tissue or organ (Lor-

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rain et al. 2002; Bodin et al. 2007), body size, diet (Fry et al. 1999), growth rate (Trueman et al. 2005), and tissue catabolism (Kurle and Worthy 2001; Olive et al. 2003).

Groupings, statistical analyses, and comparisons with the Ecopath model

Sixty-five taxa, representing 19 of the 27 ecological groups included in the Ecopath model, were analysed for stable isotopes (Table 1). Sediments resemble detritus in the Ecopath model, which by definition assigns a trophic level of 1 to detritus. Number of analysed taxa within each ecological group varied from 1 to 11 (Table 1). The ecological groups were compared within the frames of four main groups: fish, pelagic invertebrates, benthic invertebrates, and macroalgae–sediments.

Comparisons of $\delta^{13}C$ and $\delta^{15}N$ signatures were made among the different taxa within each ecological group and among ecological groups within each main group (Table 1). A separate variance *t* test that does not require equal variances was used for comparisons of two independent samples. For pairwise comparisons within several groups, a one-way analysis of variance (ANOVA), followed by Tukey's post hoc test, was used. Both tests are robust when sample sizes within each group are equal or nearly equal and when isotope signature data within a restricted interval are considered (*Zar* 1999). All tests were performed in SYSTAT (Systat Software Inc., Chicago, Illinois), with $\alpha = 0.05$.

The Sørfjord Ecopath model is a 27-compartment mass-balance model of the food web (Pedersen et al. 2008) that was accomplished without any knowledge of the results from the stable isotope analyses. The model includes the main pelagic and demersal or benthic species present in the fjord ecosystem, in addition to components of the microbial food web. Most groups are composite groups of species with similar or assumed similar diets (with dietary data mainly obtained from gut analyses), trophic positions, and life histories. Local data from Sørfjord and adjacent fjord areas were used as input. The model is based on mass-balance principles, assuming that the production of a given group is equal to the biomass lost to fishing export, predation, and natural mortality other than predation during a defined period. The Sørfjord food web model was balanced for the period 1993–1996; time was expressed as annual averages, while mass was expressed in g C·m^{-2} (Pedersen et al. 2008).

Comparisons between the stable isotopes and Ecopath methods were made at the ecological group level, with the focus on comparing trophic levels, where TL_E denotes trophic level estimated by the Ecopath model and TL_N is trophic level estimated by the isotope method. To assess the correlation between the two approaches, the average TL_N for each ecological group were plotted against the corresponding TL_E and analysed by regression analysis.

In the Ecopath model, fractional trophic levels are estimated for each ecological group by a routine that assigns trophic level 1 to producers and a trophic level of 1 + the weighted average of the prey's trophic level to consumers. Since the $\delta^{15}N$ values of the analysed taxa were used to estimate their respective TL_N, the TL_N of an ecological group was estimated from a $\delta^{15}N$ value averaged from the analysed taxa. Some of the analysed taxa are more abundant than other, and their relative contributions in terms of biomass were accounted for when ecological group averages were calculated (Table 1). The contributions were based on data from fish catches (Pedersen et al. 2008), Van Veen grab samples from Sørfjord (Nilsen et al. 2006), trawl catches and triangular dredges from Sørfjord, and grab samples from Ullsfjord (M. Nilsen, unpublished data). The relative proportions of macroalgae species were obtained from Pedersen et al. (2008). However, all these contributions were only based on the relations among the sampled taxa, and other species than those sampled may also be important. For invertebrate species in particular, only a small selection of the potential species were collected.

Results

Stable isotope composition

Average stable isotope composition of the analysed taxa ranged from –34.5‰ to –15.6‰ for $\delta^{13}C$ and from 4.2‰ to 16.1‰ for $\delta^{15}N$. As expected for "primary producers", $\delta^{15}N$ values of macroalgae and sediments varied only slightly, while large differences in $\delta^{13}C$ made it more difficult to agree on an average benthic carbon value (see next section). Benthic invertebrates showed the highest variation in ^{15}N enrichment, and the majority of the benthic taxa were also enriched in ^{13}C (Table 1, Fig. 3). There was only a moderate variation in $\delta^{13}C$ values of pelagic invertebrates, while, in spite of the analyses of few taxa, a relatively high variation was shown in ^{15}N enrichment. Fish were the most likely top predators and were also most enriched in ^{15}N, and although the $\delta^{13}C$ values varied, most values were relatively high (Table 1, Fig. 3).

$\delta^{13}C$

A more than 3.6‰ difference in $\delta^{13}C$ values between the pelagic primary producers (–24.9‰, back-calculated from *C. finmarchicus*) and all the alternatives suggested as representative for the benthic carbon (–21.1‰, –16.4‰, –21.3‰, and –19.9‰ for macroalgae, weighted macroalgae, sediment, and cod diet, respectively) makes it is possible to discriminate between a predominantly pelagic and benthic carbon source. However, the differences between the benthic carbon alternatives made it difficult to agree on a single representative value for this source. The similarity between macroalgae and sediments suggest that macroalgae is an important detritus source, but higher $\delta^{13}C$ values in many benthic consumers suggest that more enriched carbon sources are important in the system (Table 1). Since the weighted macroalgae average was dominated by one species with unknown value as food source (Table 1), this was not assumed to be representative as a major carbon source. Based on this, it was assumed that the value back-calculated from cod diet (–19.9‰) best integrated the different benthic carbon sources, and this value was therefore used as the benthic carbon average in the mixing model. The mixing model showed that benthic carbon was most important in the diet of benthic invertebrates, ranging from 53% for detrivore polychaetes to 100% for small molluscs and detrivore echinoderms. Pelagic carbon was the main source for herring (74%), while carbon originating from the benthos represented 74% and 68% for large and small cod, respectively (Table 1).

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Fig. 3. $\delta^{13}C$ and $\delta^{15}N$ (‰) values (mean ± SD) for the analysed taxa from the Sørfjord food web, grouped into (*a*) fish, (*b*) pelagic invertebrates, (*c*) benthic invertebrates, and (*d*) macroalgae–sediment. Average $\delta^{13}C$ values for pelagic (–24.9‰) and benthic (–19.9‰) carbon sources are shown by dotted and dashed vertical lines, respectively. The red algae *Phycodrys rubens* and *Ptilota serrata* had very low $\delta^{13}C$ values and were excluded from the figure.

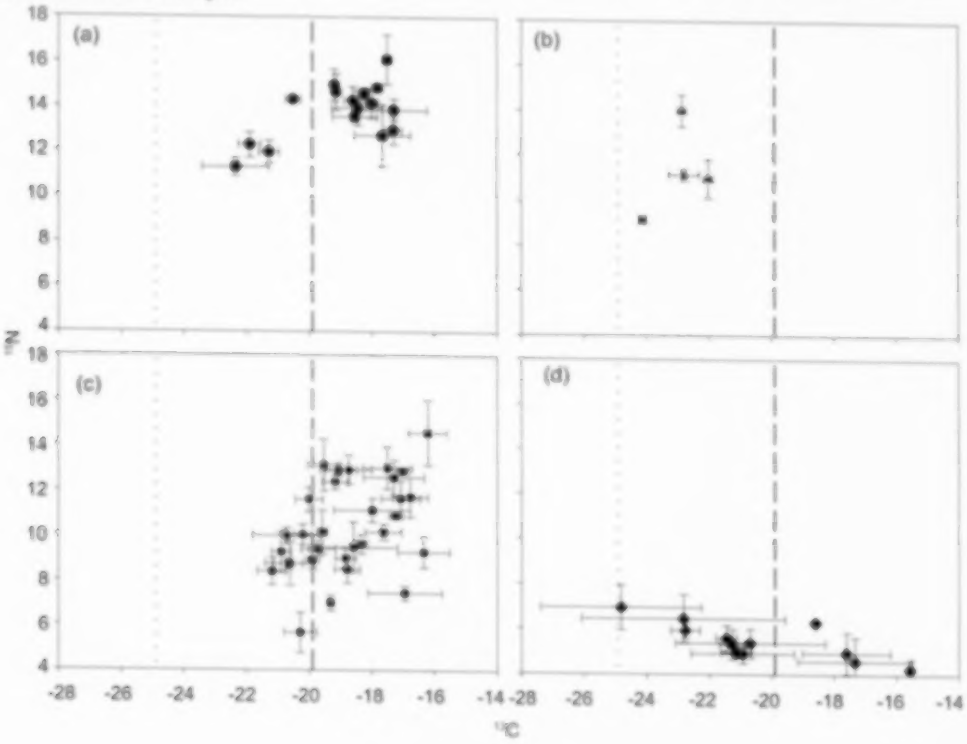
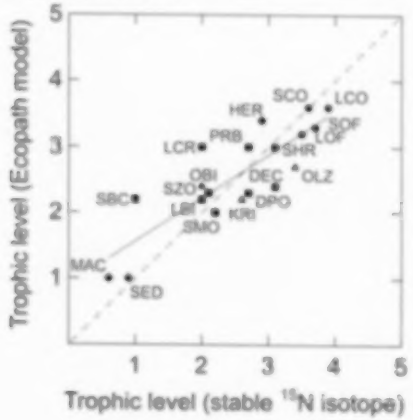


Fig. 4. Trophic levels for the ecological groups (abbreviations given in Table 1) estimated from stable ^{15}N isotopes (TL_N) plotted against their corresponding levels estimated by the Ecopath model (TL_E). Different symbols are given for ecological groups representing fish (circles), pelagic invertebrates (triangles), benthic invertebrates (squares), and macroalgae–sediment (diamonds). The regression that best fits the data is given as a solid line, and the 1:1 line is shown by a broken line.



Different composition of species within the ecological groups, and different ecology between the different species results in differences in isotope values both within and between groups. Small other fish and herring were more depleted in ^{13}C than the other fish groups ($F_{[4,52]} = 19.8$, $p < 0.001$), and all three subgroups of large cod differed from one another ($F_{[2,7]} = 166.9$, $p < 0.001$, Table 1). Small zooplankton (*C. finmarchicus*) was more depleted than the other pelagic invertebrates groups ($F_{[2,10]} = 22.8$, $p < 0.001$) and was also the most ^{13}C-depleted consumer (–24.1‰, Table 1). Small molluscs, detrivore echinoderms, and shrimp were the most ^{13}C-enriched benthic invertebrates, while other benthic invertebrates, small benthic crustaceans, and detrivore polychaetes were most depleted ($F_{[8,102]} = 10.9$, $p < 0.001$) (Table 1). The detrivore echinoderm *Ctenodiscus crispatus* was the most enriched (–16.2‰) benthic taxa (Table 1).

$\delta^{15}N$ and TL_N estimates

Of all the analysed taxa, large cod was the top predator, significantly more ^{15}N-enriched than the other ecological fish groups ($F_{[4,52]} = 8.3$, $p < 0.001$, Table 1) except small cod. However, only one trophic level separated large cod from herring (the fish group with the lowest trophic level, Table 1). Large cod individuals ranged from 39 to 115 cm in length, and cod > 70 cm had the highest TL_N (4.2) of all "taxa", while *B. glaciale* was the most depleted fish taxa ($F_{[4,12]} = 21.1$, $p < 0.001$) with the lowest TL_N (Table 1).

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Table 2. Trophic levels estimated by the stable ^{15}N isotope (TL_N) in other ecosystems for various taxa corresponding to the ecological

	Ecosystem			
	Barrow Strait – Lancaster Sound	Prince William Sound	Estuary and Gulf of St. Lawrence	North Water Polynia
Baseline	POM	*Neocalanus cristatus*	POM	*Calanus hyperboreus*
TL_N	1.00	2.00	1.00	2.00
$\Delta\delta^{15}N$ (‰)	3.8	3.4	3.4	3.8
Ecological group				
LCO			4.3	
SCO			3.8	
LOF		3.9	4.2	3.8
SOF		3.2	3.6–4.0	3.6
HER		3.3	3.5–3.8	
KRI		2.7	2.6–3.1	2.9
SZO	2.0	2.0	2.5	2.0
SHR	3.4	3.1	3.5	3.4
OLZ	2.6–2.7	2.9	2.4	2.5–2.6
LCR			3.4–3.8	
PRB	2.6–3.8		2.8	2.5–3.6
DPO				
SBC	2.3–3.6			2.3–3.4
SMO	2.4			1.8–3.0
LBI	1.7–2.2		1.8–3.1	
DEC	2.1–2.2		2.0	2.1–3.3
OBI				2.3–3.4
MAC	1.0			
PPL	1.0		1.0	

Note: Ecological group abbreviations are explained in Table 1. PPL denotes phytoplankton or POM and was not analysed in Sørfjord. $\Delta\delta^{15}N$ is the Sound, Hobson and Welch (1992); Prince William Sound, Kline and Pauly (1998); Estuary and Gulf of St. Lawrence, Lesage et al. (2001); North Water Newfoundland and Labrador shelf, Sherwood and Rose (2005); Fram Strait; Hop et al. (2006); northwestern Barents Sea, Tamelander et al. (2006).

Although only a few taxa were analysed (Table 1), the pelagic invertebrates include both the other large zooplankton, which were most enriched ($F_{[3,16]} = 47.4$, $p < 0.001$) and clearly predatory (TL_N 3.4), and the small zooplankton, which with the baseline level of 2.0 set for *C. finmarchicus* had the lowest TL_N.

The benthic invertebrates include both truly herbivore and predatory species, which was reflected in the large interval of $\delta^{15}N$ values (5.7‰ to 14.6‰, Table 1). Small benthic crustaceans (*Gammarus* sp.) were the most ^{15}N-depleted ($F_{[8,102]} = 7.2$, $p < 0.001$), while shrimp, predatory benthos, and detrivore echinoderms were most enriched (Table 1, Fig. 4). In the latter group, *C. crispatus* was the most ^{15}N-enriched taxon ($F_{[4,14]} = 43.1$, $p < 0.001$) and strongly influenced the group average (69% in biomass, Table 1). As opposed to this, the low biomass contribution (2%) of the scallop *Pseudamussium septemradiatum* resulted in an insignificant contribution to the average TL_N of large bivalves, although it was 3‰–4‰ (~1 TL_N) more enriched in ^{15}N than the other taxa ($F_{[4,13]} = 54.6$, $p < 0.001$, Table 1).

The six polychaete taxa were categorized as either detrivore polychaetes or predatory benthos (Kirkegaard 1992 1996) prior to the stable isotope analyses (Table 1). Maldanidae indeterminate dominated the detrivore biomass (65%) and was 4.7‰ more ^{15}N-enriched than *Myriochele oculata* ($t = 6.3$, df = 3, $p = 0.008$) (Table 1). The predatory polychaetes *Nephtys* sp. ($TL_N = 3.1$) and *Lumbrineris fragilis* ($TL_N = 3.2$) were more enriched than the other predatory taxa ($F_{[6,18]} = 13.0$, $p < 0.001$, Table 1), with TL_N values almost one level higher than the other polychaetes *Eunice norvegica* and Polynoidae indeterminate. (Table 1).

Comparison of stable isotopes and mass-balance model results

There was a good correlation ($F_{[1,17]} = 42.9$, $r^2 = 0.72$, $p < 0.001$) amongst the trophic level estimates of ecological groups estimated by the stable isotope method (TL_N) and the Ecopath model (TL_E), and the regression line did not differ significantly from the 1:1 line (Fig. 4).

The general trend was that TL_N values were slightly higher at high trophic levels and lower at low trophic levels compared with TL_E. This trend persisted even if the same enrichment factor were applied for all taxa, which means that it is not an artefact of the application of different enrichment factors for fish and invertebrates. The most diverging groups were small benthic crustaceans and large crustaceans, where the TL_N estimates were lower than TL_E, and the detrivore echinoderms and other large zooplankton, where the TL_N estimates were higher than TL_E (Table 1, Fig. 4).

Discussion

Carbon sources

To obtain information on all the different carbon sources in the Sørfjord food web, phytoplankton and benthic microalgae should be sampled and sediment traps deployed during spring bloom. However, microzooplankton, detritus, and macroalgae-derived particulate organic matter (POM) can

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groups in Sørfjord.

North Sea	Northern Baffin Bay	Western Norway	Newfoundland and Labrador shelf	Fram Strait	Northwestern Barents Sea
Bivalves	*Calanus hyperboreus*	Phytoplankton	Invertebrates	POM	POM
2.5	2.00	1.00	2.3	1.00	1.00
3.4	3.8	3.8	3.4 (cod 2.8)	3.4	3.4
5.2			3.3		
			3.3		4.0
4.0–4.7			3.3		
			2.8–3.4		3.5–4.0
3.8			2.7		3.4
			2.3	2.1–3.1	2.1–2.6
	2.0–3.0			2.1–3.2	2.2–2.6
	3.4		2.1–3.3		3.0–3.2
	2.5		1.4–2.7		1.9–2.7
		2.9	1.7–2.5		3.1
		2.4	3.3		2.6–3.8
					3.0
	3.1–3.4	1.5			
2.5	2.0	1.2–2.5			2.8–3.3
2.5					2.7
	3.1–3.3	1.7–2.1	1.9		2.0–3.3
		1.2–1.3			
		1.0		1.0	1.0

trophic level enrichment factor applied in the respective studies. References for data from ecosystems are as follows: Barrow Strait – Lancaster Polynia, Hobson et al. (2002); North Sea, Jennings et al. (2002); northern Baffin Bay, Fisk et al. (2003); western Norway, Fredriksen (2003);

easily contaminate phytoplankton samples in fjords (Fredriksen 2003; Søreide et al. 2006*a*), and representative $\delta^{13}C$ values can be difficult to obtain even during spring. Terrestrial plants are normally more depleted in ^{13}C than phytoplankton (Fry and Sherr 1984), and with no evidence of this from the $\delta^{13}C$ values of the analysed taxa, this carbon must either have been consumed during the summer season or is not important in this system. The pelagic carbon value from Sørfjord (–24.9‰) corresponds with the value ($\delta^{13}C$ = –24.4‰) Fredriksen (2003) used for phytoplankton in western Norway. That *C. finmarchicus* was the most ^{13}C-depleted (hence most herbivorous) pelagic consumer supports the baseline level set from a pure phytoplankton diet. There are large uncertainties related to the average value of benthic carbon. Since this was more enriched than the sediments and most macroalgae, pelagic carbon may mistakenly be interpreted as more important than it really is for sediment-ingesting detrivore species. On the other hand, Nadon and Himmelmann (2006) found elevated $\delta^{13}C$ values in benthic consumers and suggested that this was caused by other factors or processes than the feeding of benthic primary producers, such as high input of POM during periods of high productivity, rework, and degradation of particles during sinking or selective feeding on enriched POM. This means that the importance of benthic, relative to pelagic, primary production may have been overestimated for benthic consumers in several studies, including this one. Extremely high $\delta^{13}C$ values as observed in benthic omnivores and detrivores by Hobson et al. (2002) was not seen in Sørfjord. Although relatively high carbon ratios were seen for some groups, this suggests that fresher carbon and detritus sedimenting from the pelagic zone is more important in this system.

Pelagic and benthic life strategies are also reflected in the $\delta^{13}C$ enrichment among and within fish groups. Small other fish is a composite group with both pelagic and benthic species represented, and this explains the observed differences in ^{13}C enrichment. For the purpose of analysing carbon sources, composite groups should be split also according to habitat. Herring is a pelagic species with a weaker connection to the pelagic environment in Sørfjord than in Newfoundland and on the Labrador Shelf, where 100% of the carbon was of pelagic origin (Sherwood and Rose 2005). Low $\delta^{13}C$ values (–20.3‰, Tamelander et al. 2006) also indicate a more pelagic feeding in the Barents Sea compared with the Estuary and Gulf of St. Lawrence (–18.8‰, Lesage et al. 2001). However, with different carbon sources, in addition to temporal and spatial variability, direct comparisons of $\delta^{13}C$ values from different ecosystems should be made with care.

The benthic community in the outer (Tarehola) area of Sørfjord has a higher abundance of large, mobile epifauna compared with the small-sized, more typical soft bottom fauna in the inner areas (Njosken, Nilsen et al. 2006). The fact that cod feed in more benthic zones in Tarehola than in Njosken may therefore be explained by the higher abundance of preferred benthic prey. Enriched $\delta^{13}C$ values in large cod indicate either a more benthic diet or a higher trophic level diet, and although not supported by statistics, the

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latter explanation is the most likely. A switch from a more benthic to a more piscivore diet at larger size in Sørfjord was also observed by Kanapathippillai et al. (1994). The low pelagic input to the cod diet suggests that pelagic krill was less important in 2006 than during the model period (1993–1996), when krill was highly abundant and constituted 50% of the diet (Pedersen et al. 2008). The benthic inputs are high compared with other ecosystems (Fredriksen 2003; Sherwood and Rose 2005) and reflect an opportunistic feeding strategy, where cod switch between different preys based on availability. The similarity in isotope patterns among the dominating demersal fishes like cod and long rough dab suggests that they depend on both pelagic and benthic trophic pathways in Sørfjord.

Trophic levels, comparisons with Ecopath, and suggestions for improvements

The good correlation between TL_N and TL_E support the diet composition that was used for most groups in the Ecopath model, and this resembles results from other studies where the two methods have been compared (Kline and Pauly 1998; Mathisen and Sands 1999; Dame and Christian 2008). With the majority (19 of 27) of the ecological groups in the Sørfjord Ecopath model represented with isotope data, this makes the good correlation more impressive, although future effort should be expended to obtain isotope data also for the remaining groups. In Prince William Sound, Kline and Pauly (1998) found a very good correlation between the two methods, with points distributed about the 1:1 line, when comparing seven ecological groups. They also applied their isotope data to another ecosystem, the Alaskan Gyre. Although the precision was reduced, it was still good, which suggests that isotope results can be transferred between ecosystems. Hence, the isotope data obtained from Sørfjord will be valuable also for the future modelling of other high-latitude ecosystems. Dame and Christian (2008) used stable isotopes to validate model outputs, and when validation was not met, they used the isotopes to determine the effects of modifying trophic networks to reflect this validation. In the following, we attempt to explain the deviances between the two methods and propose future improvements to the construction of ecological groups in ecosystem models.

Trophic levels estimated by stable isotope analyses are subjected to methodological implications related to pretreatment, trophic fractionation of $\delta^{15}N$ and the choice of isotopic baselines (Post 2002; Carabel et al. 2006; Søreide et al. 2006*b*). To explore the effects of other choices of trophic enrichment factor ($\Delta\delta^{15}N$) and baseline ^{15}N ($\delta^{15}N_{base}$), a simple sensitivity analysis was undertaken. A scenario with a low $\Delta\delta^{15}N$ of 2.3‰ as suggested by McCutchan et al. (2003) and a low $\delta^{15}N_{base}$ of 8.0 calculated as an average of potential baseline taxa at trophic level two (*Myriochele oculata*, *Arctica islandica*, *Chlamys islandica*, *Modiolus modiolus*, *Musculus niger*, *Strongylocentrotus droebachiensis*, and *Gammarus* sp.) resulted in high correlation with TL_E (r^2 = 0.70) but with an increase in TL_N of about 0.9 and thus larger differences from TL_E for the higher ($TL_N > 2.5$) groups. The enrichment factors used in this study were amongst the lowest (3.4‰ for most organisms, 3.2‰ or 2.9‰ for fish) in a comparison between studies (Table 2). A second sensitivity scenario with a high $\Delta\delta^{15}N$ of 3.8‰ for all groups as suggested by some other studies (Table 2) and $\delta^{15}N_{base}$ of 9.0‰ also gave high correlation between TL_E and TL_N (r^2 = 0.70), but gave lower TL_N of the high trophic level groups that was more similar to TL_E. Between-ecosystem comparisons will also be influenced by the choice of $\Delta\delta^{15}N$ and $\delta^{15}N_{base}$, but the values used in the different studies were similar and all enrichment factors were higher than the value of 2.3‰ suggested by McCutchan et al. (2003).

The selection of *C. finmarchicus* as a baseline consumer with a TL_{base} of 2.0 resembles the approaches of Hobson et al. (2002) and Fisk et al. (2003), who used the same level for *Calanus hyperboreus* (Table 2). Although *C. finmarchicus* is regarded a primary herbivore, other matter, such as microbial organisms, may be ingested (Pasternak et al. 2001) and potentially increase TL_{base}. To account for this, one possibility would be to adjust the TL_{base} to 2.4 to resemble the TL_E of *C. finmarchicus*, and this would increase the remaining TL_N estimates by 0.4.

A benefit with the stable isotope method is that it provides time-integrated diet information. Turnover rates of different tissues are, however, not constant but depend on factors such as life span and type of tissue, and changes in diet are reflected most rapidly in metabolically active tissues (Lorrain et al. 2002; Bodin et al. 2007). Temporal changes in feeding rates can also change isotope signatures (Olive et al. 2003). Many high-latitude organisms, including copepods (Pasternak et al. 2001), maintain their winter metabolism by utilizing stored lipids and carbohydrates, which may increase their tissue $\delta^{15}N$ (Kurle and Worthy 2001; Olive et al. 2003). This implies that the $\delta^{15}N_{base}$ obtained from *C. finmarchicus* is likely to correspond to a higher TL_N than 2.0, since samples were collected during winter. Also, since the Ecopath model was balanced on data representing 1993–1996 and the isotope data represent 2005–2007, the time lag could reflect real changes in the ecosystem.

The long-lived large bivalves can integrate temporal diet variation in the isotopic signatures, and this makes them suitable as baselines in mature ecosystems. The average large bivalve TL_N of 2.0, with *C. finmarchicus* as baseline, is similar to the baseline level selected for the filtering scallop *Pecten maximus* by Pinnegar et al. (2002), while Jennings et al.'s (2002) baseline level (2.5) was an average of several bivalve species. However, the large variation in TL_N estimates for filtering bivalves in this study (1.8–3.1) and in studies by Lesage et al. (2001) and Tamelander et al. (2006) (all using other baselines) show that robust baseline assumptions are difficult to make for this group of species.

Comparisons between the stable isotope and the Ecopath methods were made among ecological groups. In most cases, isotope analyses were made from a relatively small selection of species or taxa originally included in the Ecopath ecological groups (Pedersen et al. 2008). Since species are not evenly distributed, and some are more abundant than others, some discrepancies were accounted for by considering the relative contribution to biomass of the analysed taxa when TL_N was estimated for ecological groups. The limited number of analysed taxa was a result of high sampling and processing costs, in addition to chance during sampling. Although the correlation between the methods was good, an improved correspondence between the two trophic level esti-

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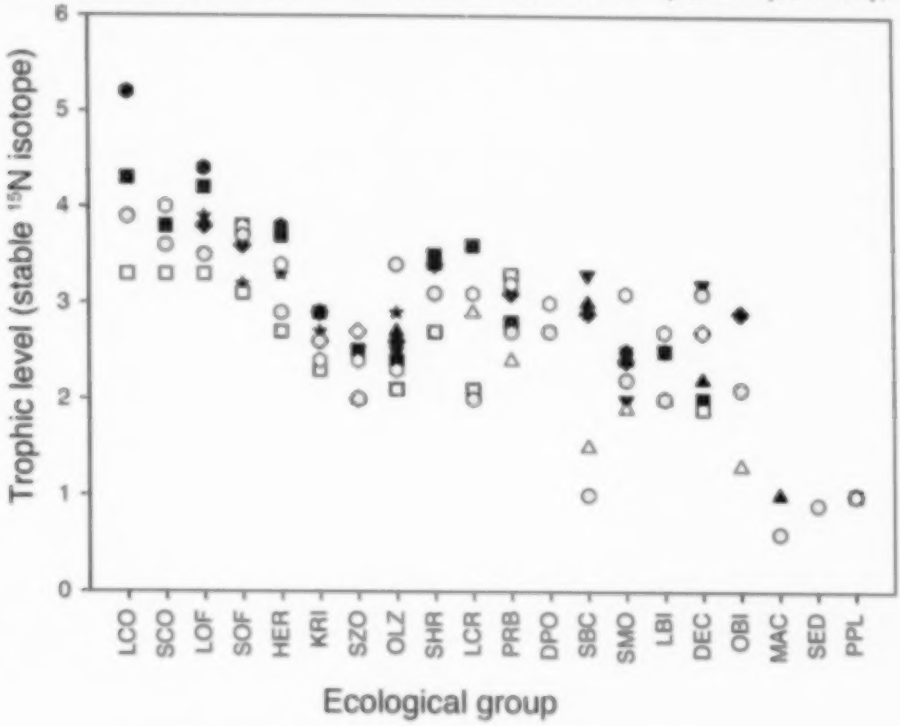
Fig. 5. A comparison of different ecosystems, based on literature review (see Table 2), of trophic levels estimated from stable ^{15}N isotopes (TL_N). For other ecosystems than Sørfjord, data from Table 2 are averaged, and the importance of taxa are not accounted for. Abbreviations are given in Table 1. PPL denotes phytoplankton or particulate organic matter (POM) (not analysed in the present study).



mates would probably be accomplished for some of the groups if more species within the group were analysed. The largest differences between the analysed and potential number of taxa were found for the benthic invertebrate groups, which also showed the largest differences between the trophic level estimates.

With no analyses made from mammals and birds that were the top predators in the Ecopath model (Pedersen et al. 2008), cod obtained the highest TL_N estimates. The TL_N for cod from Sørfjord corresponded well with TL_E, but were lower than that for cod in other ecosystems (Table 2, Fig. 5), a pattern also seen for TL_E (Pedersen et al. 2008). The applied enrichment factor inflates the cod TL_N by ~0.1 to 0.2 trophic level relative to the other estimates (except those from Sherwood and Rose 2005; Table 2) and thus does not explain the lower TL_N estimates. Only a small part of the difference from North Sea cod (TL_N = 5.2; Jennings et al. 2002) would be explained by increasing the baseline level in Sørfjord by 0.5 trophic level (according to Jennings et al. 2002), and there are probably real differences between these systems. Hence, if isotope results are transferred between ecosystems, this should not be made without some basic knowledge on the comparability of the systems in advance.

Large crustaceans and predatory asteroids often occupy higher trophic levels in benthic marine food webs. The fact that TL_N for large crustaceans was approximately one trophic level lower than TL_E was surprising, since the analysed species also dominated the Ecopath group (Pedersen et al. 2008). Higher TL_N estimates are also observed in other ecosystems (3.1 for *Pagurus pubescens* in the Barents Sea (Tamelander et al. 2006) and 3.4–3.8 for other small crab species in the Estuary and Gulf of St. Lawrence (Lesage et al. 2001)). Relative small-sized *Hyas* were analysed in this study, and the discrepancies may be explained by a switch from a more herbivore and detrivore feeding at small size to a more predatory and scavenging feeding, more similar to the Ecopath diet (Pedersen et al. 2008), at larger size. Also, lower $\delta^{15}N$ values are observed in exoskeleton compared with muscle (Gorokhova and Hansson 1999), and the analyses of whole individuals may have lowered the TL_N estimate relative to TL_E. One possibility is therefore to split

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more groups according to size if large dietary changes or changes in vulnerability to predation are expected as they grow.

Some surprisingly large differences between TL_N and TL_E were observed among the benthic invertebrates at the bottom, or assumed bottom, of the food web. The TL_N estimated for small benthic crustaceans was not only the lowest but also, where the two estimates diverged, the most. Only one amphipod taxa (*Gammarus* sp.) was analysed and chance plays a major role, although amphipods are the major group also in the Ecopath model group (Pedersen et al. 2008). The TL_N may have been underestimated because exoskeletons were included in the analysed material (Gorokhova and Hansson 1999), but the low $\delta^{15}N$ value resembles the value measured for *Gammarus locusta* feeding on degraded macroalgae (Norderhaug et al. 2003). Other large zooplankton was also represented by only one amphipod species, *Hyperia galba*, in the analyses. This parasitic hyperiid amphipod has a carnivorous diet (Dahl 1959; Dittrich 1992), which explains the high TL_N, both compared with TL_E and TL_N estimates from other ecosystems (Table 2, Fig. 5). Analyses of more representative and higher number of taxa for these composite groups would possibly yield TL_N values that were both closer to TL_E and more comparable with other ecosystems. The construction of separate carnivorous and herbivorous groups to account for different feeding strategies within diverse ecological groups should also be considered in future modelling.

Another assumed low trophic level group is the detrivore echinoderms. The surprisingly high TL_N (owing to *C. crispatus*) is robust to changes in the enrichment factor and the baseline level, suggesting that the TL_N is valid. This is also supported by high TL_N values (2.6–3.3) in other ecosystems (Hobson et al. 2002; Fisk et al. 2003; Tamelander et al. 2006). Lipid analyses have shown that bacteria and other meio- and micro-organisms can contribute substantially to their diets (Sargent et al. 1983), which suggest that micro- and meio-fauna are more important in Sørfjord than previously assumed (Pedersen et al. 2008). This is supported by the enriched ^{15}N of another assumed detrivore, the polychaete Maldanidae indeterminate (Kirkegaard 1992), which also is similar to the TL_N of 3.0 estimated for *Maldane sarsi* in the Barents Sea (Tamelander et al. 2006). Ecopath automatically assigns trophic level 1 to detritus (Christensen and Pauly 1992), whereas others (Burns 1989) argue that the trophic level of detritus should be that of the organism that released it, which would probably give a better correspondence between the two trophic levels for detrivore groups.

The composition of the ecological groups in the Ecopath model did not account for the fact that some species are not preferred in the diet of fish and other higher trophic level organisms, resulting in a low direct contribution to the classical food web. For one of these species, *C. crispatus*, this is supported by both the high TL_N relative to fish and the few observations made of this species in fish stomachs. Given the existence of benthic groups that are partly decoupled from the classical food web, an alternative is to treat these organisms (e.g., *C. crispatus*; the sea cucumber *Cucumaria frondosa*; and the largest predatory asteroids, *Asterias rubens*, *Crossaster papposus*, and *Solaster endeca*) as separate groups to emphasize their importance and ecosystem function.

When ecosystems are modelled, there is always a trade-off between simplicity and complexity, and accuracy is not necessarily based on the latter. Species are aggregated to reduce the number of model compartments, and although effort is made to create natural groups, there will be within-group variability. Kline and Pauly (1998) showed that isotope data could be transferred amongst ecosystems when used as a comparison with the mass-balance model output, though with reduced accuracy. This indicates that the isotopic results from this study may be helpful to explain patterns and design models in other similar ecosystems. Tanabe and Namba (2005) conclude that an understanding of the stabilizing and destabilizing role of omnivory in ecosystems requires better knowledge about the relative contributions of the different resources in the omnivore's diet and how they may change. The variable isotope results illustrates the lack of knowledge of the diet of many benthic invertebrates, and for systems like Sørfjord where these organisms are important, this is an important task that requires continued work.

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PERSPECTIVE / PERSPECTIVE

Prevalence and recurrence of escaped farmed Atlantic salmon (*Salmo salar*) in eastern North American rivers

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Abstract: Knowledge of the prevalence of escaped farmed fishes in the wild is an essential first step to assessing the risk resulting from interactions between farmed and wild fishes. This is especially important in eastern North America, where Atlantic salmon (*Salmo salar*) aquaculture occurs near wild Atlantic salmon rivers and where many wild salmon populations are severely depressed. Here, we review the literature on the incidence of escaped farmed salmon in eastern North American rivers, for which there has been no comprehensive compilation to date. Escaped farmed salmon have been found in 54 of 62 (87%) rivers investigated within a 300 km radius of the aquaculture industry since 1984, including 11 rivers that contain endangered salmon populations. Averaged among all investigations, the proportional representation of farmed salmon among adults entering the rivers from the sea was 9.2% (range 0% to 100%). Where data were sufficient to examine temporal trends, farmed salmon proportions varied considerably over time, suggesting that escape events are episodic in nature. We conclude that escaped farmed salmon are sufficiently prevalent in eastern North American rivers to pose a potentially serious risk to the persistence of wild salmon populations, especially in those rivers that are adjacent to existing aquaculture sites.

Résumé : Une évaluation de la prévalence des poissons échappés de pisciculture en nature est un premier pas essentiel dans la détermination des risques reliés aux interactions entre les poissons d'élevage et les poissons sauvages. La situation est particulièrement sérieuse dans l'est de l'Amérique du Nord où des cultures de saumons atlantiques (*Salmo salar*) se retrouvent près de rivières à saumons atlantiques sauvages et où plusieurs des populations de saumons sauvages sont considérablement réduites. Nous faisons ici une revue de la littérature sur l'incidence de poissons échappés de culture dans les rivières de l'est de l'Amérique du Nord, dont il n'existe pas à ce jour de compilation exhaustive. On a trouvé des saumons échappés de culture dans 54 des 62 (87 %) rivières étudiées dans un rayon de 300 km de l'industrie piscicole depuis 1984, dont 11 rivières contenant des populations de saumons en voie de disparition. Dans l'ensemble des inventaires, la proportion moyenne de saumons de pisciculture parmi les saumons qui pénètrent dans les rivières à partir de la mer est de 9,2 % (étendue: 0 % à 100 %). Dans les cas où les données sont assez nombreuses pour permettre d'étudier les tendances temporelles, les proportions de saumons de pisciculture varient considérablement dans le temps, ce qui laisse croire que les incidents de fuites sont de nature épisodique. Nous concluons que la prévalence de saumons échappés de pisciculture est suffisamment grande dans les rivières de l'est de l'Amérique du Nord qu'elle représente une menace pour la persistance des populations de saumons sauvages, particulièrement dans les rivières qui sont à proximité de sites actuels d'aquaculture.

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Introduction

Worldwide declines in wild fish stocks combined with a growing human population have resulted in increased demands for aquaculture production. Over 25% of the world's fish consumption is now of aquaculture origin, and aquacul-

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ture has been increasing at an average annual rate of 8.8% since 1970, higher than the growth rate of both capture fisheries and terrestrial animal agriculture (Naylor et al. 2000; UN Food and Agriculture Organization (FAO) 2006). The aquaculture of farmed Atlantic salmon (*Salmo salar*) is no exception to this trend. In the North Atlantic alone, farmed salmon production amounted to 817 100 tonnes (t) in 2006, a 171-fold increase in production since 1980 (International Council for the Exploration of the Sea (ICES) 2007). By comparison, the total North Atlantic nominal catch of wild salmon in 2006 was only 0.24% of its aquaculture counterpart (ICES 2007). Farmed salmon now considerably outnumber their wild conspecifics within the native range of the species (Gross 1998; Hansen et al. 1999; Hansen and Windsor 2006).

Despite preventative measures, farmed salmon escape into the wild from sea cages and juvenile hatcheries in most, if not all, regions where they are farmed; they have also been found in areas where farming does not occur. Escaped farmed salmon have been reported in, among other places, Iceland (Gudjonsson 1991), Norway (Gausen and Moen 1991; Lund et al. 1991; Fiske et al. 2006), Scotland (Youngson et al. 1997; Walker et al. 2006), Northern Ireland (Crozier 2000), the Republic of Ireland (Walker et al. 2006), England and Wales (Milner and Evans 2003; Walker et al. 2006), the Faroe Islands (Hansen and Jacobsen 2003), Greenland (Hansen et al. 1997), Chile (Soto et al. 2001), Turkey (Innal and Erk'akan 2006), Australia (The Australian 2007), the United States (Baum 1998; Lage and Kornfield 2006), and Canada (Carr et al. 1997; Volpe et al. 2000). Damage to sea cages (from storms, vandalism, or predators) has been identified as the principal cause of several major escape events, including 600 000 adult farmed salmon that escaped from one farm in a single event near the Faroe Islands in 2002 (see McGinnity et al. 2003). Many escapes go unreported. The "leaking" of juveniles from freshwater hatcheries comprises a source of underreported farmed salmon escapes (Carr and Whoriskey 2006). One recent estimate suggests that two million farmed salmon escape every year into the North Atlantic Ocean (Schiermeier 2003).

Although escaped farmed salmon are thought to incur initially high mortalities in the wild (Whoriskey et al. 2006), both adult and juvenile farmed escapees can still enter rivers in large numbers (Lacroix and Stokesbury 2004; Fiske et al. 2006). Here, they may interbreed with wild salmon, despite somewhat poorer reproductive success relative to wild salmon (Fleming et al. 1996, 2000; Weir et al. 2004). This has raised concerns about the potential detrimental effects that interactions between farmed and wild salmon can have on wild populations. For instance, artificial selection, random genetic drift, and the use of non-native stocks result in farmed salmon escapees that are genetically distinct from their wild counterparts (Mjolnerod et al. 1997; Roberge et al. 2006; Hutchings and Fraser 2008). Based on the prediction that wild salmon populations are locally adapted to their particular environments (Garcia de Leaniz et al. 2007), farmed–wild interbreeding could lead to a reduction in fitness in farmed–wild hybrids (Hutchings 1991; McGinnity et al. 2003; Fraser et al. 2008). In addition, farmed salmon may transmit disease (McVicar 1997), competitively displace wild salmon (Fleming et al. 2000), superimpose spawning nests over those of wild salmon (Lura and Sægrov 1991; Webb et al. 1993), and increase the uncertainties associated with stock assessments of wild salmon (Hansen et al. 1999).

A critical first step to assessing the risk that escaped farmed salmon might pose to wild salmon populations is to quantify the frequency with which farmed salmon enter wild salmon rivers and the frequency with which such escapes recur. Farmed salmon escapees have been relatively well documented in parts of Europe (Lund et al. 1991; Hansen et al. 1999; Fiske et al. 2006). However, a similar compilation of their occurrence has not been undertaken for rivers in eastern North America (NA). This is despite the fact that much of the salmon aquaculture industry in eastern NA is concentrated in the same geographic location as the most endangered wild Atlantic salmon populations on the continent (Committee on the Status of Endangered Wildlife in Canada (COSEWIC) 2006; US Atlantic Salmon Assessment Committee (US ASAC) 2006).

Most salmon farming in eastern NA occurs in the Canadian provinces of Newfoundland and Labrador (NL), New Brunswick (NB), and Nova Scotia (NS) and in the US state of Maine (ME), with ME and NB accounting for >80% of the total production (Fig. 1). Freshwater aquaculture hatcheries are often situated along rivers that contain genetically distinct and phenotypically unique wild salmon populations (King et al. 2001; Fraser et al. 2007*a*, 2007*b*), and sea cages are in close proximity to the estuaries of those rivers. Since the inception of the North American salmon farming industry in 1980, the production of farmed salmon has dramatically increased in all farming areas (Fig. 1). In 2005, eastern NA produced 51 000 t of finfish (the vast majority being salmon) worth more than CAN$305 million (Statistics Canada 2006; S. Horn Olsen, Aquaculture Policy Coordinator, Maine Department of Marine Resources, 21 State House Station, Augusta, ME 04333-0021, personal communication) and comprising 4% of global Atlantic salmon production.

Notwithstanding the absence of a comprehensive database, there is reason to believe that farmed salmon escape events may pose a threat to wild salmon populations. For example, more than 300 000 farmed salmon adults are estimated to have escaped over a 6-year period (2000–2005) in ME and NB (F.G. Whoriskey, unpublished data). Despite these escapes, most published data in the primary scientific literature have been collected from a single salmon river, NB's Magaguadavic River, where the proportion of farmed salmon in two years exceeded 90% (Carr et al. 1997). This river is located in one of the world's most intensive salmon farming regions and has been systematically monitored for escapes for this reason. The extent to which this river's data can be applied to other eastern NA rivers for risk assessment is unknown. In addition, although organizations and government agencies in both Canada and the US have been collecting information on the presence of farmed salmon in several major rivers for many years, much of this information is scattered and uncollated.

In an attempt to build a comprehensive database on the escapes of farmed salmon in eastern NA, we collated all available information from both primary and secondary sources on the presence of farmed salmon in ME, NB, NL, and NS rivers from 1980 (the year in which salmon farming

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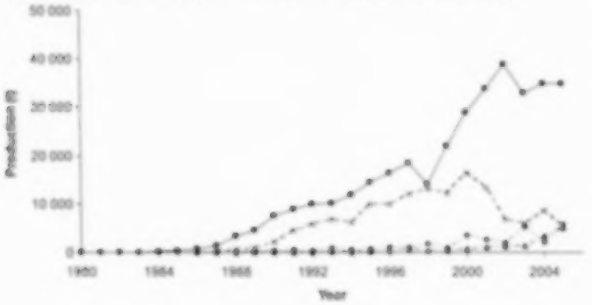
Fig. 1. Annual production of farmed Atlantic salmon (*Salmo salar*) for eastern North America: Nova Scotia (◆), New Brunswick (■), Newfoundland (▲), Maine (×). Adapted from data from Newfoundland and Labrador Department of Fisheries and Aquaculture (2006) and Maine Department of Marine Resources (2006).



commenced in NA) through 2006. We had four major objectives: (*i*) to review and summarize available information on farmed salmon escapes from sea cages and aquaculture hatcheries in eastern NA; (*ii*) to determine the number of rivers in eastern NA in which the presence or absence of farmed salmon has been investigated, including rivers harbouring endangered wild salmon populations as defined under the US Endangered Species Act (ESA) and Canada's Species At Risk Act (SARA); (*iii*) to quantify, where possible, the proportion of escaped farmed salmon relative to wild salmon in rivers where farmed salmon have been detected; and (*iv*) to broadly assess whether the proportional representation of farmed salmon in wild salmon rivers varies over time.

Materials and methods

Literature search procedure

To quantify the incidence of farmed salmon in eastern NA rivers, we undertook a literature search for primary, peer-reviewed journal articles, using Web of Science and the Google Scholar search engines. Major contributing authors of these papers, as well as their reference sections, were also searched to identify additional publications of interest.

We also undertook an extensive literature search for primary or secondary reports on the occurrence of farmed salmon in eastern NA as produced by organizations such as the Atlantic Salmon Federation (ASF) (www.asf.ca), Committee on the Status of Endangered Wildlife in Canada (COSEWIC) 2006 (www.cosewic.gc.ca), Fisheries and Oceans Canada (DFO) (www.dfo-mpo.gc.ca), International Council for the Exploration of the Sea (ICES), including the Working Group on North Atlantic Salmon (www.ices.dk), Atlantic Salmon Commission (ASC) (www.maine.gov/asc), National Marine Fisheries Service, including the Northeast Fisheries Science Center and the US Atlantic Salmon Assessment Committee (USASAC) (www.nmfs.noaa.gov), North Atlantic Salmon Conservation Organization (NASCO) (www.nasco.int), US Fish and Wildlife Service (www.fws.gov), and the World Wildlife Fund (WWF) (www.worldwildlife.org). For Canadian provinces, this included a review of any DFO stock assessment papers, research papers, working papers, and meeting minutes that pertained to farmed Atlantic salmon, as listed on the Canadian Science Advisory Secretariat Web site (www.dfo-mpo.gc.ca/csas). Salmon stock assessment reports were available for most years between 1984 and 2006 for either specific rivers or regions within eastern Canada. For Maine, the ASC's official reports to the ME legislature were acquired for the 2001–2004 period. Members of the ASC were contacted and tables, figures, and stock assessments created by the USASAC were provided for the years 1998–2005. Additional publications that may have been missed by directly searching all of the above-mentioned Web sites were subsequently located by searching Google and Google Scholar, using specific rivers or researchers as the search terms.

In total, 116 references were used to compile Table 1 and Supplementary Tables S2–S4 (this list of references is available from the NRC Depository of Unpublished Data[2]). Depending on the nature of the source (primary or secondary literature) and the level of detail reported in the study, individuals identified as the primary researchers for studies on particular river systems were in some instances contacted and additional information was requested. Sometimes unpublished information pertaining to additional rivers was also provided. The sources of these data are cited here as "personal communications".

Data compilation and definitions

Information pertaining to farmed and wild salmon for the years in which the presence of farmed salmon had been investigated were compiled from the above sources. Relevant data pertaining to farmed salmon in each eastern NA river were collated in tabular form. These data included information on (*i*) known escape events, (*ii*) numbers of farmed salmon caught, (*iii*) numbers of wild salmon caught (hatchery-released fish are included in the wild counts), (*iv*) size or age class of the farmed salmon, (*v*) occurrence of European genes in the farmed salmon, (*vi*) methods used to capture or observe the wild and farmed salmon, (*vii*) means used to determine whether the salmon were farmed or wild, (*viii*) evidence of hybridization between farmed and wild salmon, and (*ix*) reasons for why data may not have been acquired for a particular year in a particular river.

There were many instances in which secondary sources were used. In some cases, the origin of the data used by the secondary source had not been identified or cited. In these circumstances, we contacted the authors of the secondary source and the necessary information was often obtained. In at least 17 instances, the secondary sources cited data from unpublished personal correspondence with a field biologist (e.g., data for the Bear and Tusket rivers (NS) in O'Neil et al. (2005)). In such cases, the biologists were contacted to confirm the data; if there was no response (8 of 17 researchers) and based on the high accuracy of the other similar instances that we verified with the biologists, the information

[2] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 3868. For more information on obtaining material refer to cisti-icist.nrc-cnrc.gc.ca/cms/unpub_e.html.

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Table 1. Incidence of farmed Atlantic salmon (*Salmo salar*) adults in eastern North American rivers.

River	P or S[a]	Year	Total no. of wild salmon adults	Total no. of farmed salmon adults	Total no. of salmon adults	Farmed salmon adults (%)	Count method[b]	ID[c]
Annapolis	NS	1990–1996		1				
		1995		?				
		1998	13	1	14	7	A,S	
		1999		?				
		2000		15				
Baddeck	NS	1994	231	1	232	0.4	M&R	
		1995	338	23	361	6	M&R	EX
		1995	146	8	154	5	SC	FE
		1996	214	0	214	0	SC	EX
		1997	137	1	138	0.7	SC	EX
		1998	190	5	195	3	M&R	
		1999	N/A	N/A	N/A	N/A		
		2001	N/A	N/A	N/A	N/A		
Bear	NS	Early 1980s		Many			A	
Bras d'Or Lakes	NS	1994		Detected				
Gaspereau	NS	1990–1996		5				
		1992	23	1	24	4		
		1993	8	3	11	27		
		1994	35	0	35	0		
		1995	35	1	36	3		EX
		1996		?	178	<3 ?	FW	
		1997		?		<3 ?	FW	
		1998	27	1	28	4	FW	
		2000	~49	1	~50	2	FW	
		2004		0		0		
		???		Detected				
Grand	NS	2003		0		0		
Harrington	NS	2003		Suspected				
Indian Brook	NS	2000		1				
LaHave	NS	1989	3 176	1	3 177	0.03		
		1990–1994		0		0		
		1995	499	0	499	0		
		1996	422	0	422	0		
		1997	314	0	314	0		
		1998	1 880	0	1 880	0	FW	
Liscomb	NS	1994–1998	984	0	984	0		
Margaree	NS	1987–1996	>2 000	0	>2 000	0		
Mersey	NS	2000	5	1	6	17	A	
Meteghan	NS	2000		1				
Middle	NS	1994		?	475		M&R	
		1995	183	0	183	0	SC	EX
		1996	358	1	359	0.3	SC	EX
		1997	258	0	258	0	SC	EX
		1998	213	9	222	4	M&R	
Nictaux	NS	1996	38	1	39	3	D	EX,SA
		1999	5	0	5	0	FW	
		2000	5	12	17	71	FW	
North	NS	1995	154	14	168	8		
		1995	178	3	181	2	SC	
		1996	322	0	322	0	SC	FE
		1997	335	0	335	0	SC	EX
		1998	433	55	488	11	SC,M&R	
		1999	N/A	N/A	N/A	N/A		
		2000	N/A	N/A	N/A	N/A		
		2003	N/A	N/A	N/A	N/A		
Parrsboro	NS	???		Released				
Sackville	NS	1996–1998	515	0	515	0		
Salmon (Digby)	NS	1999		2				
		2000		0		0		
Skye	NS	1998		?	71		A,S	
Stewiacke	NS	1995	14	?	21	33	CF,EF	SA

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Table 1 (*continued*).

River	P or S[a]	Year	Total no. of wild salmon adults	Total no. of farmed salmon adults	Total no. of salmon adults	Farmed salmon adults (%)	Count method[b]	ID[c]
		1996	8	0	8	0	EF	EX
		1997		0		0		
St. Ann's Bay	NS	1998		?				
St. Mary's	NS	1992–1993, 1997–1998	796	0	796	0		
Tusket	NS	1998	>158	2	>160	~1	FW	
Whycocomagh Bay	NS	1994	116	2	118	2	HT	
		1995	34	147	181	81	HT	
		1996	10	40	50	80	HT	
		1997	N/A	N/A	N/A	N/A		
Belle Isle Bay	NB	1990		Several	280		HT	
Big Salmon	NB	Late 1980s		1				
		2000	11	0	11	0		
		2001	44	0	44	0	SH,SC	
		2002	33	0	33	0	SH,SC	
		2004		0		0		
Black River	NB	2003		Detected		*		G
Bocabec	NB	1999	0	2	2	100	CF	
Chamcook Stream	NB	17–23 Nov. 2005		5				
Dennis Stream	NB	2001		Detected				
Magaguadavic	NB	1983	889	51	940	5	FW	
		1984	783	0	783	0	FW	
		1985	635	0	635	0	FW	
		1988	689	0	689	0	FW	
		1983–1988		0		0		
		1990–1996		2 301			FW	
		1992	294	147	441	33	FW	FE,FL,SA
		1993	237	154	391	39	FW	FE,FL,SA
		1994	131	1 198	1 329	90	FW	FE,FL,SA
		1995	79	712	791	90	FW	FE,FL,SA
		1996	69	240	309	78	FW	FE,FL,SA
		1997	59	119	178	67		FE,SA
		1997	32	8	40	20	FW	SA
		1998	31	227	258	88	FW	EX,SA
		1999	24	90	114	79	FW	EX,SA
		1999, 2000	30	35	65		FW	EX,SA,G
		2000	14	30	44	68	FW	FE,SA
		2001	17	132	149	89	FW	FE,SA
		2002	7	35	42	83	FW	FE,SA
		2003	6	23	29	79	FW	FE,SA
		2004	2	17	19	89	FW	FE,SA
		Up to 13 Oct. 2005	9	38	47	81	FW	
		15 Nov. 2005		6				
		17–23 Nov. 2005		30				
		2005	9	69	78	88	FW	FE,SA
		2006	26	6	32	19	FW	
Miramichi	NB	1980s – 1998		0	1000s	0		
Mitchell Brook	NB	17–23 Nov. 2005		6				
Nashwaak	NB	1997	736	0	736	0	CF	
		1998	1 565	0	1 565	0	CF,M&R	FE
		1999	940	0	940	0	CF,M&R	FE
		2000	700	0	700	0	CF,M&R	
		2001	516	0	516	0	CF	
		2002	422	0	422	0	CF	FE,SA
		2005	521	1	522	0.2	CF	SA
Saint John	NB	1990	12 700	229	12 929	1.8	FW	

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Table 1 (*continued*).

River	P or S[a]	Year	Total no. of wild salmon adults	Total no. of farmed salmon adults	Total no. of salmon adults	Farmed salmon adults (%)	Count method[b]	ID[c]
		1991	13 886	80	13 966	0.57	FW	
		1992	13 788	50	13 838	0.36	FW	
		1993	7 752	6	7 758	0.08	FW	
		1994	5 881	28	5 909	0.47	FW	FE
		1995	7 328	106	7 434	1.4	FW	
		1996	10 031	13	10 044	0.13	FW	
		1997	5 226	0	5 226	0	FW	FE
		1998	5 876	4	5 880	0.07	FW	
		1999	4 983	20	5 003	0.4	FW	FE,BF
		2000	3 568	6	3 574	0.17	FW	
		2001	2 859	14	2 873	0.49	FW	
		2002	2 702	13	2 715	0.47	FW	
		2003	2 021	3	2 024	0.15	FW	
		2004	2 167	1	2 168	0.05	FW	
		2005	1 490	0	1 490	0	FW	
		2006	1 662				FW	
St. Croix	NB	1990–1996		231				
		1992	N/A	N/A	N/A	N/A		
		1994	84	97	181	54	FW	
		1995	47	13	60	22	FW	
		1996	132	20	152	13	FW	
		1997	43	27	70	39	FW	
		1998	42	25	67	37	FW	EX
		1999	13	23	36	64	FW	EX
		2000	20	30	50	60	FW	
		2001	21	56	77	73	FW	
		2002	20	6	26	23	FW	
		2003	15	9	24	38	FW	
		2004	10	4	14	29	FW	
		17–23 Nov. 2005		3				
		2005	6	33	39	85	FW	FE,SA
		2006	4	7	11	64		
Upper Salmon	NB	2003		Spawning adults				G
Waweig	NB	1980s		1				
Conne	NL	1990–1996		3				
		1993		1				SA
		1994	92	2	94	2.1		SA
		1995	120	0	120	0		SA
		1996	97	0	97	0		SA
		1997	422	8	430	1.9		EX
		1997	233	2	235	0.9		SA
		1997	476	4	480	0.8		SA
		1998	189	2	191	1	CF	SA
		1998		1			A	
		1999	173	1	174	0.6	CF	BF,SA
		2000	216	5	221	2.3		SA
		2001	189	0	189	0	CF,A	EX,SA,I
		2002	279	0	279	0		SA
		2003	181	0	181	0		SA
		2004	264	0	264	0		SA
		2005	203	0	203	0		SA
		2006		0		0	CF	EX
Little	NL	1994	84	0	84	0	CF	
		1995	135	0	135	0	CF	
		1996	801	0	801	0	CF	
		1997	478	0	478	0	CF	
		1998	311	2	313	0.6	CF	
		1999	351	5	356	1.4	CF	
		2000	616	0	616	0	CF	

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Table 1 (*continued*).

River	P or S[a]	Year	Total no. of wild salmon adults	Total no. of farmed salmon adults	Total no. of salmon adults	Farmed salmon adults (%)	Count method[b]	ID
		2001	161	0	161	0	CF	
		2002	528	0	528	0	CF	
		2003	335	0	335	0	CF	
		2004	687	0	687	0	CF	
Androscoggin	ME	2000	4	0	4	0		
Boyden Stream	ME	???		Detected				
Cobscook Bay	ME	2004		273				
Cobscott Bay	ME	???		Detected				
Dennys	ME	1993–1996		67				
		1993	40–50	20	60–70	29–33		EX,SA
		1994	5	42	47	89	W	EX,SA
		1995	5	4	9	44	W	EX,SA
		1996	10	21	31	68	W	EX,SA
		1997	0	2	2	100		EX,SA
		1998	1	1	2	50	W	EX,SA
		1999	N/A	N/A	N/A	N/A		
		2000	2	28	30	93	W	
		2001	25	65	90	72	W	
		2002	2	4	6	67	W	
		2003	9	2	11	18	W	SA
		2004	1	0	1	0	W	SA
		2005	0	8	8	100	W,GN,A,EF	
		2006	5	4	9	44	W	
East Machias	ME	1990	69	14	83	17	A	
		1995–1999		Detected			A	
		1998	0	0	0		A	
		1999	0	0	0		A	
		2000	N/A	N/A	N/A	N/A		
		2001	N/A	N/A	N/A	N/A		
		2002	N/A	N/A	N/A	N/A		
		2003	N/A	N/A	N/A	N/A		
		2004	N/A	N/A	N/A	N/A		
		???		Detected				G
Hobart Stream	ME	???		Detected				
Machias	ME	1998	5	0	5	0	A	
		1999	0	0	0		A	
		2000	N/A	N/A	N/A	N/A		
		2001	N/A	N/A	N/A	N/A		
		2002	N/A	N/A	N/A	N/A		
		2003	N/A	N/A	N/A	N/A		
		2004	N/A	N/A	N/A	N/A		
Narraguagus	ME	1994	51	1	52	2	FW	
		1995–1996		9				
		1995	56	0	56	0	FW	
		1996	28	8	36	22	FW	
		1997	37	0	37	0	FW	
		1998	22	0	22	0	FW	
		1999	32	3	35	9	FW	
		2000	23	0	23	0	FW	
		2001	32	0	32	0	FW	
		2002	8	0	8	0	FW	
		2003	21	0	21	0	FW	
		2004	11	0	11	0	FW	
		2005	6	0	6	0	FW	
		2006	5	0	5	0	FW	
Pennamaquan	ME	???		Detected				
Penobscot	ME	1990		1				
		1994–2001				<0.01		
		1994		0				G
		1995		0				G
		1996		0				G

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Table 1 (*concluded*).

River	P or S[a]	Year	Total no. of wild salmon adults	Total no. of farmed salmon adults	Total no. of salmon adults	Farmed salmon adults (%)	Count method[b]	ID[c]
		1997	~1 077	0	~1 077	0		
		1998	1 210	0	1 210	0	FW	
		1998		0				G
		1999	968	0	968	0	FW	
		1999		0				G
		2000	535	0	535	0		
		2000		5				G
		2001	785	1	786	0.1	FW	
		2001		9				G
		2002	776	4	780	0.5	FW	
		2002		0				G
		2003	1 114	0	1 114	0	FW	
		2003		0				G
		2004	1 323	0	1 323	0	FW	
		2005	985	0	985	0	FW	
		2006	1 046	1	1 047	0.1		
Pleasant	ME	1997	N/A	N/A	N/A	N/A		
		1998	0	0	0		A	
		1999	0	0	0		A	
		2000	3	0	3	0	W	
		2001	11	0	11	0	W	
		2002	0	0	0		W	
		2003	2	0	2	0	W	
		2004	1	0	1	0	W	
		2005–present	N/A	N/A	N/A	N/A		
		???		Detected		2		G
Togus Stream	ME	???		Detected				G
Union	ME	1994–1998		N/A		N/A		
		1999	1	10	11	91	FW	SA
		2000	2	3	5	60	FW	
		2001	0	2	2	100	FW	SA
		2002	5	6	11	55	FW	
		2003	1	0	1	0	FW	
		2004	2	0	2	0	FW	SA
		2005	0	4	4	100	FW	SA
		2006		0		0		
Unknown	ME	1991, 1992		Few				
		2005		Many				

Note: Question marks (???) in the Year column indicate that a source did not report the year but provided data on numbers of farmed salmon, and we could not find a way to assign the values to a specific year. A single question mark (?) means that the sources were difficult to interpret but ambiguously suggested the presence of farmed salmon in a river in a particular year. The 116 references, as well as footnotes and 1SW/MSW (1 sea winter/multiple sea winters) salmon numbers, are found in Supplementary Table S1. Parr and smolt information is in Supplementary Tables S2–S3 (available online from NRC Data Depository).

[a]Province (P) or state (S): NS, Nova Scotia; NB, New Brunswick; NL, Newfoundland and Labrador; ME, Maine.

[b]A, angling; CF, counting fence; D, driftnet; EF, electrofishing; FW, fishway; GN, gill net; HT, monitoring-harvest trap; M&R, mark and recapture; S, seining; SC, snorkel count; SH, shore count; W, weir.

[c]Method used to identify farmed or wild origins of salmon: BF, body form; EX, external characteristics; FE, fin erosion; FL, fork length; G, genetics; I, isotopes; SA, scale analysis.

in the secondary source was assumed to be accurate. Note that secondary sources are not differentiated from primary sources in Table 1 and the supplementary tables.

If a literature source only provided limited information on a certain river, such as the total number of salmon caught in a particular year and the percentage of those that were of farmed origin, we calculated and reported estimates of the number of farmed and wild salmon from the information presented. These estimates are indicated by a "~" in front of the salmon numbers in Table 1 and in the supplementary information. If a source did not report the year but provided data on numbers of farmed salmon and we could not find a way to assign the values to a specific year, we placed a "???" in the date column. If the reports indicated only that farmed salmon were taken but not the actual numbers caught, we scored this as "detected" in the farmed salmon column. Some sources speculated on the presence of farmed salmon in a particular river based on anecdotal evidence; in such cases, the river was mentioned and the term "suspected" was placed in the farmed salmon column. Quotes of the speculations from the source reference are provided in a supplementary footnote (Supplementary Appendix S1,

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available online from NRC Data Depository[2]). In a few instances, the sources were difficult to interpret but ambiguously suggested the presence of farmed salmon in a river in a particular year; this was indicated by "?" under the farmed salmon column and the appropriate quote was again footnoted.

If researchers specifically mentioned the presence or absence of farmed salmon in a river in a given year, that river was classified as "investigated" for that particular year. Investigated rivers included rivers in which trained personnel verified that no farmed salmon were present. All investigated rivers were included in Table 1 and Supplementary Tables S1–S3 (available online from NRC Data Depository[2]). If researchers did not mention the presence or absence of farmed salmon in a river in a given year, it was assumed that there were no trained personnel present to identify farmed salmon at that river and the river was classified as "uninvestigated" for that particular year. Uninvestigated rivers were not included in Table 1 or the supplementary tables, with the exception of the "suspected" rivers as defined above.

Farmed salmon were distinguished from wild salmon in the literature based on the following methods: (*i*) body morphology and fin erosion (reviewed in Jonsson and Jonsson 2006), (*ii*) scale analysis (as per Lund and Hansen 1991; Stokesbury et al. 2001; Lacroix and Stokesbury 2004), (*iii*) genetic screening (Skaala et al. 2004; O'Reilly et al. 2006), (*iv*) isotope analysis (Dempson and Power 2004), and (*v*) age at smolting (Carr 2005). These methods, when known, are identified in Table 1 and the supplementary tables.

For the purposes of this paper, a salmon "adult" is defined as any salmon above the smolt life stage, including immature postsmolts. The term "parr" is used in reference to the posthatch freshwater juvenile phase of the salmon life cycle, and "smolts" are postparr salmon that have undergone physiological changes prior to seaward migration. "Farmed" adults are salmon that (*i*) escaped directly from sea cages, (*ii*) escaped from freshwater aquaculture hatcheries as parr or smolts and survived to adulthood, or (*iii*) were the adult offspring of sea cage or hatchery escapees that spawned in the wild. The first type of farmed adult is likely the type most commonly referenced in Table 1; type *ii* fish could only be identified by scale, isotope, or genetic analyses and type *iii* fish could only be identified by genetic or isotope analyses. Any adult visually identified as farmed could only be, if correctly classified, a sea cage escapee. Farmed parr and farmed smolts were, unless otherwise noted, escapees from freshwater aquaculture hatcheries situated along the rivers in which they were found; the few exceptions include the offspring of farmed salmon that had successfully spawned in the wild.

In many wild salmon rivers, salmon are reared in hatcheries and released to supplement the wild population. If a source identified a salmon adult as "hatchery origin", they were grouped with the wild salmon (Table 1; with the notable exception of the Little River, NL, reports in which "hatchery" was a euphemism for farmed escapee). This decision was made in part because farmed salmon are often moved to geographic locations that contain genetically divergent wild salmon populations, whereas hatchery salmon are generally raised along their ancestral rivers (Hutchings and Fraser 2008). Also, unlike hatchery salmon, farmed salmon undergo several generations of artificial selection (Roberge et al. 2006), further diverging farmed salmon from wild salmon. Our decision to group hatchery origin salmon with wild salmon met its greatest challenge in the Saint John River (NB) in which wild, farmed and hatchery fish are all of Saint John River origin and thus cannot be positively identified based on genetic screening (P. O'Reilly, Fisheries and Oceans Canada, 1 Challenger Drive, Bedford Institute of Oceanography, Dartmouth, NS B2Y 4A2, Canada, personal communications). However, in this river, hatchery and farmed salmon could be distinguished from wild salmon based on fin erosion and scale analysis, and farmed salmon could further be distinguished from hatchery salmon based on scale analysis and the presence of an adipose fin, as all hatchery fish were fin-clipped (Jones et al. 2004). Hatchery-released fish are also adipose fin clipped in the Magaguadavic River, NB (Carr 2005). Although these methods may not always correctly classify the salmon as "farmed" or "hatchery–wild", they are accurate to a high degree and we accepted the reports of the field researchers as accurate with some potential caveats (see Discussion).

The total number of salmon found in a river in a particular year was based, whenever possible, on actual counts of the salmon spawning run. In some circumstances, salmon abundance was estimated using mark–recapture techniques. In those cases, the number of farmed salmon was sometimes based on an estimate provided by the mark–recapture study and sometimes was a minimum estimate based on the actual number found. The minimum values probably underestimate the true numbers of farmed salmon present. The use of mark–recapture data is indicated (Table 1). There were also some instances (such as with the Conne River, NL) where a subset of the total run was sampled and the proportion of farmed salmon within that sample was recorded. In these cases, the sample size and not the spawning run size was included in the table; this is indicated by a footnote in the supplementary information.

Statistics and figures

Some simple summary statistics of the information presented in Table 1 and the supplementary tables are provided in the Results section and accompanying figures. These calculations include the following: mean proportions of farmed salmon by region, river, and year; the proportion of investigated rivers that contained farmed salmon; the "detection rate", defined as the percentage of investigations that detected farmed salmon; and search effort, defined as the number of rivers that were investigated in a particular year. Because many data points were incomplete (a "data point" being a row in Table 1), some had to be excluded from these calculations.

The mean proportions of adult farmed salmon were calculated from the proportions of farmed salmon within individual rivers for individual years. Only those data points for which proportions and years were given were used (Table 1). All bays (Belle Isle, Cobscott, Cobscook, St. Ann's, and Whycocomagh) were excluded from these calculations, as were suspected or questionable rivers. For the LaHave, Liscomb, Margaree, Miramichi, Sackville, and St. Mary's riv-

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ers, each year in which no farmed salmon were found was considered an independent data point, with an equal number of wild salmon assumed for each year. The Miramichi data were assumed to begin in 1989, and only one data point was assumed for the Bear River. All other data points that encompassed multiple years were excluded. In some instances, several data points were available for a single river for the same year; Table 1 has been constructed such that the data point used in these calculations is the first one mentioned. Data points that covered an entire year were favoured over those that encompassed only a few days. If a range of possible proportions was provided, the highest proportion was used. Finally, all data points were excluded from the proportion calculations for which the numbers of both wild and farmed salmon were zero, as they could not properly be said to be a proportion. Thus, although the East Machias was investigated for three years, only the one year in which any salmon were found is included in these calculations. Six data points were excluded for this reason.

The regional (province or state and salmon fishing area) and yearly mean proportions of farmed salmon in the salmon spawning run were calculated using a modified version of Freeman and Tukey's (1950) arcsine transformation (Anscombe 1948; Zar 1999). This transformation corrected for differences in the numbers of farmed and wild salmon found among rivers and accounted for the high frequency of proportion values in our data set that approached either 0 or 1 (see Zar 1999). The range (the lowest and highest proportions in the untransformed data set) was provided along with the transformed means. Only those data points (n = 196) that fulfilled the above conditions and contained both the number of wild salmon and farmed salmon were used.

The proportion of rivers that contained farmed salmon were calculated by adding all of the investigated rivers that contained farmed salmon in at least one year and dividing by the total number of investigated rivers. Suspected and questionable rivers were excluded from these calculations, as were Cobscook Bay (because farmed salmon were intentionally released into it) and Bras D'Or Lakes (as it is not separate from Whycocomagh Bay). All other water systems were included. Rivers for which the exact number of farmed salmon or the year in which they were found was unknown were also included in these calculations, as long as the presence of farmed salmon had been confirmed. Rivers that contained farmed salmon in multiple years were only counted once.

For detection rate and search effort calculations, all data points that included a year and either the number of farmed salmon or the confirmed presence of farmed salmon were included. A data point that had a proportion of farmed salmon greater than zero was scored as a "success". Again, the Bear River was treated as one data point, the Miramichi data were assumed to begin in 1989, and all of the years included in the LaHave, Liscomb, Margaree, Miramichi, Sackville, and St. Mary's rivers data sets were counted as independent data points. All other data points that encompassed multiple years were excluded, including those years scored as "detected" in the East Machias River. Cobscook Bay and Bras D'Or Lakes were excluded, but all other bays were included. If two methods were used to identify farmed salmon in the same year and one method detected farmed salmon and the other did not, the one that detected farmed salmon was used as a data point, providing all other conditions were met.

There were several cases in which information from one document conflicted with information from another. In those cases, the primary or peer-reviewed source is displayed in Table 1 and the supplementary tables. If no primary source was found (or if primary sources conflicted), the source that contained more data (e.g., the number of farmed salmon and the total number of salmon, as opposed to only the percentage of farmed salmon) was used. All conflicting information is indicated in footnotes in the supplementary tables. If possible, explanations are suggested to explain the conflicting data. The figures and calculations that follow in our review use the data from Table 1 and exclude any conflicting information cited in the supplementary footnotes.

Results

Frequency and magnitude of farmed salmon escapes in eastern North America

We found 27 reports of documented farmed salmon escapes from sea cages and aquaculture hatcheries in eastern NA since 1980 (Table 2). The main causes of farmed salmon escapes (when reported) were storms (n = 8) and vandalism (n = 8), followed by gear failure (n = 2), boating accidents (n = 1), and handling error (n = 1). Although three of these escapes were very large (n = >100 000), even the smallest (n = 200) was over half the size of the spawning run of wild salmon in the adjacent river for that year.

We hypothesized that the frequency and magnitude of farmed salmon escapes increased as aquaculture production increased in eastern NA, but there was no evidence of such a relationship (Fig. 1; Table 2). Although farmed salmon production has steadily increased since 1984, and the frequency of escape events and the estimated number of farmed salmon escapees in 1994 and 2000 were comparatively higher than in 1984, the intervening years show no consistent pattern. This could be due to data deficiencies; it is likely that there were more escapes than documented.

Importantly, escape events often coincided with peaks in the numbers and proportions of farmed salmon recorded in neighboring river systems (Figs. 2, 3). For example, the escape of 20 000–40 000 salmon from a sea cage in NB in 1994 led to the highest recorded catch of farmed salmon in any river in eastern NA — nearly 1200 farmed salmon were caught in the Magaguadavic River (NB) in 1994, up from 154 in 1993. However, not every peak coincided with reported escape events. The 1997 peak for the Dennys (ME) and St. Croix (NB) rivers, the 1999 peaks for the Union (ME), St. Croix (NB), and Narraguagus (ME) rivers, and the 1995 peak for the Saint John River (NB) could not be linked to any particular reported escape event.

The proportions of adult farmed salmon within the salmon spawning run in rivers varied considerably among years (Fig. 2), with the Magaguadavic River (NB) being a notable exception. This river exhibited slight peaks; however, salmon returns to the river from the sea have been consistently dominated by farmed fish (Fig. 2).

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Table 2. Documented escapes of farmed Atlantic salmon (*Salmo salar*) adults and juveniles from sea cages and aquaculture hatcheries.

Year	Number	Life stage	Location	Cause	Reference[a]
1984	5000	1.5–3 kg	East Dover, NS	Vandalism	99
1994	20000–40000		SW NB	Storm	43, 96
1994			Eastport, ME		37
Feb. 1995	100000	Parr	NL	Storm	38
June 1995	20000		Cape Breton, NS	Vandalism	38
1996			Cape Breton, NS	Vandalism	71
1996			Libby Island, ME	Storm	45
1998	8000		Annapolis Basin, NS		43, 96
1998	63300	Parr	Jeddore Lake, NL		115
1999	50000		Annapolis Basin, NS		43, 115
1999	200		Vyse Cove, NL	Handling error	115
1999	6300	Smolts	May Cove	Gear failure	115
2000	30000 maximum		Tinkers Island, NB	Gear failure	115
2000	100000–170000	5 lb	Stone Island, ME	Storm	29, 45, 115
2000	13000	2–2.5 lb	Eastport, ME	Boat crash	115
Dec. 2000	15000	800 g	Nantucket Island, NB	Storm	115
2000 total	175000 minimum in six escape events	Salmon and rainbow trout	Eastern NA	Storms (3), vandalism (1), boat crash (1), unknown (1)	48, 49
2001	3000–5000		Eastport, ME		29
2001	6	Smolts	Deblois Hatchery, ME		45
2003	2000		Birch Cove, ME	Storm	45, 115
May 2003	6500	Adults	NL		
2004		800 g	ME	Storm	83
April–May 2005	26300	1.5 and 2.4 kg	Deer Island, NB	Vandalism	84, 115
Aug. 2005	20000	400–500 g	St. Andrew's, NB	Vandalism	84, 114, 115
Summer 2005	Tens of thousands	Adults	Cooke's Aquaculture, NB	Vandalisms (3)	20
Nov. 9, 2005	100000	4–5 kg	Cooke's Aquaculture, NB	Vandalism	21, 84, 114

[a]See Appendix S4 in Supplementary data for references (available online from NRC Data Depository).

Incidence of escaped farmed salmon in eastern North American rivers

From 1984–2006, escaped farmed salmon juveniles and (or) adults have been documented in 54 of 62 (87%) rivers investigated within a 300 km radius of the aquaculture industry in ME, NL, NB, and NS (Figs. 4, 5; Table 1; Supplementary Tables S1–S3, available online from NRC Data Depository[2]). Of these 62 rivers, adult salmon were investigated in 50; farmed adult salmon were found in 42 (84%) of these 50 rivers (Tables 1, 3). Of these 42 rivers with adult farmed salmon, estimates for 15 were based on data obtained over a period of five or more years; adult farmed salmon were detected in multiple years in 12 of 15 (80%) of these rivers. Nineteen of 22 rivers (86%) in which parr were screened had parr of farmed origin (Table 3; Supplementary Table S2, available online from NRC Data Depository[2]) and 4 of 4 rivers searched contained farmed smolts (Table 3; Supplementary Table 3, available online from NRC Data Depository[2]). Farmed salmon were not detected in eight investigated rivers (Androscoggin, Grand, Liscomb, Margaree, Miramichi, St. Mary's, and Sackville rivers, and Buckman's Creek; Figs. 4, 5; Tables 1, 3; supplementary tables). They were also suspected to occur in an additional three rivers or bays (Skye River, St. Ann's Bay, and the endangered Harrington River; Fig. 4; Table 1; see supplementary footnotes for Table 1, available online from NRC Data Depository[2]).

Within regions (NS, NB, NL, ME), farmed salmon adults have been identified in 13 of 14 rivers investigated in NB (for the purpose of this paper, the St. Croix River is considered part of NB because the monitoring station was in the NB portion of the river), 16 of 21 NS rivers, 2 of 2 NL rivers, and 11 of 13 ME rivers (Tables 1, 3). Farmed parr have been detected in 10 of 13 NB rivers and 9 of 9 ME rivers (Table 3; Supplementary Table 2, available online from NRC Data Depository[2]). The presence of farmed parr has not been reported for any NL or NS river, nor were any reports found indicating that their presence had been investigated. Similarly, farmed smolts have been documented in two NB rivers, one NL river, and one ME river, with no published results for NS (Table 3; Supplementary Table 2).

In total, whenever the presence or absence of adult farmed salmon has been investigated, there has been a detection rate of 49%, or 114 out of 235 searches conducted (i.e., the sum of the total number of years searched per river). By region, farmed salmon adults were detected 32% (8/25) of the time in NL, 43% (26/61) in ME, 39% (28/71) in NS, and 68% (53/78) in NB (Tables 1, 3).

The number of rivers in which adult farmed salmon were found in a particular year closely reflected the search effort (Fig. 6), defined here as the total number of rivers investigated in a particular year. Thus, years in which few rivers contained adult farmed salmon appeared to also be years in which few rivers were searched, and vice versa (Fig. 6). For example, in 2000, 13 rivers were found to contain adult farmed salmon out of 20 that had been searched. In 2005, adult farmed salmon were found in only half as many rivers ($n = 7$), but only about half as many rivers ($n = 11$) had been searched. An outlier year was 1997, in which effort was high ($n = 18$) but the presence of adult farmed

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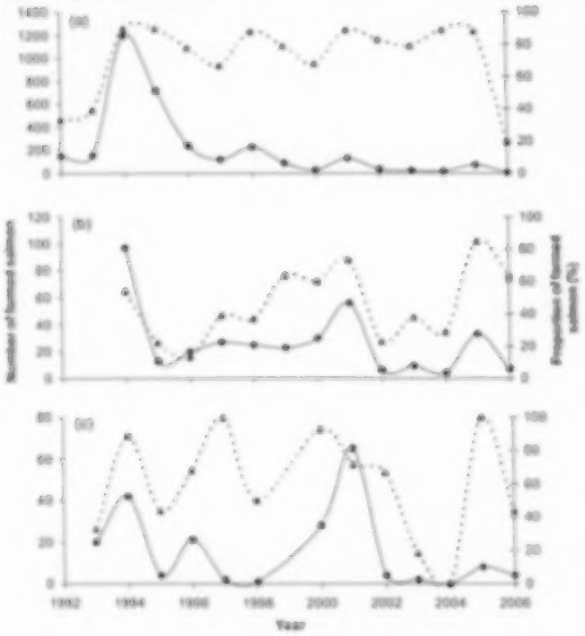
Fig. 2. Numbers (solid line) and proportions (broken line) of farmed Atlantic salmon (*Salmo salar*) over time in three river systems. Note that the numbers and proportions fluctuate from year to year, possibly correlating to mass escape events. (*a*) Magaguadavic River, New Brunswick; (*b*) St. Croix River, New Brunswick; (*c*) Dennys River, Maine.



salmon was low ($n = 5$). This may, however, be attributable to the fact that several rivers previously known to have contained farmed salmon were not searched in 1997 (Table 1).

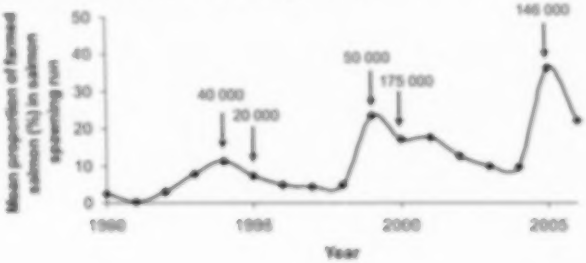
Escaped farmed salmon in rivers harbouring endangered salmon populations

Farmed salmon have been documented in 11 rivers that harbour endangered wild salmon populations as defined under the US ESA or Canada's SARA, including six of eight endangered rivers in ME (Dennys, East Machias, Machias, Narraguagus, Pleasant, and Sheepscot) that have been deemed to contain salmon that genetically correspond to historic populations. Of the approximately 40 rivers with SARA-listed endangered salmon in the Inner Bay of Fundy, Canada, all five that have been searched have been found to contain farmed salmon (Big Salmon, Gaspereau, Parrsboro, Stewiacke, and Upper Salmon rivers; see supplementary footnotes[2] for Table 1 for the Parrsboro and Harrington rivers). The endangered population with the highest reported proportions of escaped farmed salmon was the Dennys River, ME, with farmed salmon comprising 100% of the salmon spawning run in 1997 and possibly again in 2005 (only farmed salmon were found, but adult counts may have been incomplete).

Proportions and counts of escaped farmed salmon

To date, adult farmed salmon have been found in 42 rivers within a 300 km radius of the aquaculture industry in

Fig. 3. Yearly mean proportions of escaped farmed Atlantic salmon (*Salmo salar*) within the salmon spawning run, averaged over all eastern North America rivers. Peaks may correspond to mass episodic escape events. The 5 years in which it is known that more than 10 000 adult farmed salmon escaped are indicated by arrows, with the estimated number of escapees noted. Data were arcsine-transformed as per Freeman and Tukey (1950).



eastern NA (Table 1). Just over half of these rivers (22) had counts or estimates of both wild and farmed salmon returns. In these investigated rivers, for at least one year, farmed salmon contributed ≥10% of the total salmon spawning run in 12 (54%), ≥20% of the run in 9 (41%), ≥80% of the run in 5 (23%), and 100% of the run in 3 (14%) of the rivers (Table 1). Importantly, although the proportion of farmed salmon in rivers was high in some years, it fluctuated greatly from year to year, often peaking after mass escapes (Figs. 2, 3). For example, farmed salmon proportions in the Dennys River, ME, increased from 0% to 100% over consecutive years (Fig. 2). In NS, the highest recorded proportion of farmed salmon in a river occurred in the Nictaux River in 2000 when 12 of 17 (71%) of the salmon caught in the fishway were of farmed origin. The second highest was from a partial count in 1995 in the now-endangered Stewiacke River, where farmed salmon comprised 33% of the total that had been sampled. Only two rivers in NL (Conne and Little) have been examined for the presence of farmed salmon, with the higher proportion comprising just over 2% of the run. By contrast, ME and NB were characterized by the highest farmed salmon proportions, attaining 100% in ME's Dennys River (1997 and possibly 2005) and Union River (2001 and 2005) and in NB's Bocabec River (1999). Farmed salmon have contributed to more than 80% of the run in NB's Magaguadavic River in 7 of 15 years in which counts have been made, and only once since 1994 has this proportion declined below 60% (Fig. 2*c*).

Collectively, for all investigations, the mean proportion of adult farmed salmon among returning wild salmon, based on Freeman and Tukey's (1950) arcsine transformation, was 0.4% (range 0% to 2.3%) in NL, 1.8% (range 0% to 71%) in NS, 20.2% (range 0% to 100%) in ME, and 16.8% (range 0% to 100%) in NB (Table 3; Fig. 5). The overall mean proportion of adult farmed salmon in eastern NA, for all investigations, was 9.2% (range 0% to 100%). For the period of 1990–2006, the mean per year for all of eastern NA fell within the range of 0.2% (1991) to 36.3% (2005) (Fig. 3). Taken another way, the sum of the number of farmed salmon from all investigations, divided by the sum of the number of all wild and farmed salmon, yielded a proportion of escaped farmed salmon of 3.0%. However, in removing two

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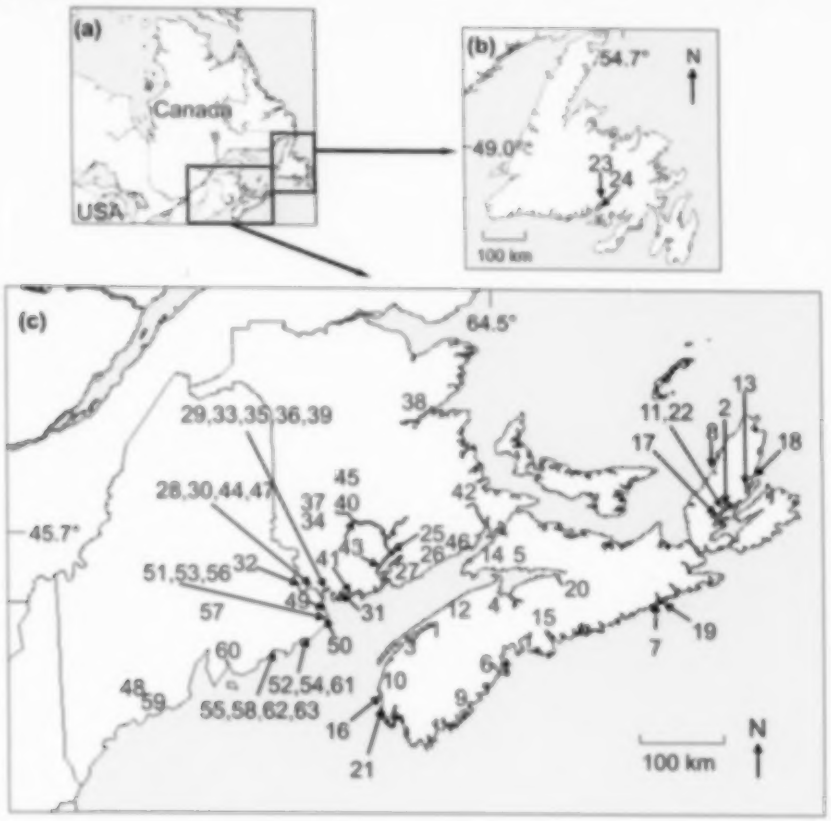
Fig. 4. Maps of (*a*) northeastern North America, (*b*) rivers in Newfoundland (NL) mentioned in Table 1, and (*c*) rivers in Maine, New Brunswick, and Nova Scotia mentioned in Table 1.



Nova Scotia (1-22)
1 Annapolis River
2 Baddeck River
3 Bear River
4 Gaspereau River
5 Harrington River
6 LaHave River
7 Liscomb River
8 Margaree River
9 Mersey River
10 Meteghan River
11 Middle River
12 Nictaux River
13 North River
14 Parrsboro River
15 Sackville River
16 Salmon River (Digby)
17 Skye River
18 St. Ann's Bay
19 St. Mary's River
20 Stewiacke River
21 Tusket River
22 Whycocomagh Bay

Newfoundland (23-24)
23 Conne River
24 Little River

New Brunswick (25-47)
25 Belle Isle Bay
26 Big Salmon River
27 Black River
28 Bocabec River
29 Buckman's Creek
30 Chamcook Stream
31 Cripp's Stream
32 Dennis Stream
33 Digdeguash River
34 Kellys Creek
35 Linton Stream
36 Magaguadavic River
37 Mill Stream
38 Miramichi River
39 Mitchell Brook
40 Nashwaak River
41 New River
42 Petitcodiac River
43 Saint John River
44 St. Croix River
45 Tay River
46 Upper Salmon River
47 Waweig River

Maine (48-63)
48 Androscoggin River
49 Boyden Stream
50 Cobscook Bay
51 Dennys River
52 East Machias River
53 Hobart Stream
54 Machias River
55 Narraguagus River
56 Pennamaquan River
57 Penobscot River
58 Pleasant River
59 Sheepscot River
60 Union River
61 Chase Mill Stream
62 Beaver Meadow Brook
63 Bog Stream

rivers (Saint John and Penobscot) that accounted for a very large fraction of the total number of wild salmon in all investigations (73.4%), the proportion of farmed salmon changed to 9.8%. This is similar to our reported mean of 9.2% using the proportions of farmed salmon within individual rivers, an approach that accounts for the small size of many wild salmon populations in the region.

In Canada, the provinces containing Atlantic salmon are partitioned into salmon fishing areas (SFAs) for management purposes. SFAs located in principal aquaculture regions include SFA 19 (eastern Cape Breton region), SFA 22 (inner Bay of Fundy), and SFA 23 (inner and outer Bay of Fundy). Investigations in these SFAs yielded mean adult farmed salmon proportions of 1.7% (range 0% to 11%), 11.1% (range 0% to 71%), and 22.6% (range 0% to 100%), respectively. For the few rivers that had been searched in

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Fig. 5. Mean proportions and numbers of adult, farmed Atlantic salmon (*Salmo salar*) in eastern North American rivers (excluding Newfoundland and Labrador): Maine, ME; Nova Scotia, NS; New Brunswick, NB. In the charts, the mean proportion of farmed salmon within the spawning run of a river for all years of available data is indicated by solid areas in each pie chart; the proportion of wild–hatchery adult salmon is indicated by open areas. The numbers above each pie chart indicate the number of years in which data were collected for a river system, and the numbers below indicate the mean number of farmed salmon found in the river system for the investigated years. Inset: location of aquaculture sites (triangles). This may include other salmonids and some sites that are not currently in operation. Inset adapted from COSEWIC (2006), Nova Scotia Fisheries and Aquaculture (2008), and Anderson (2007).

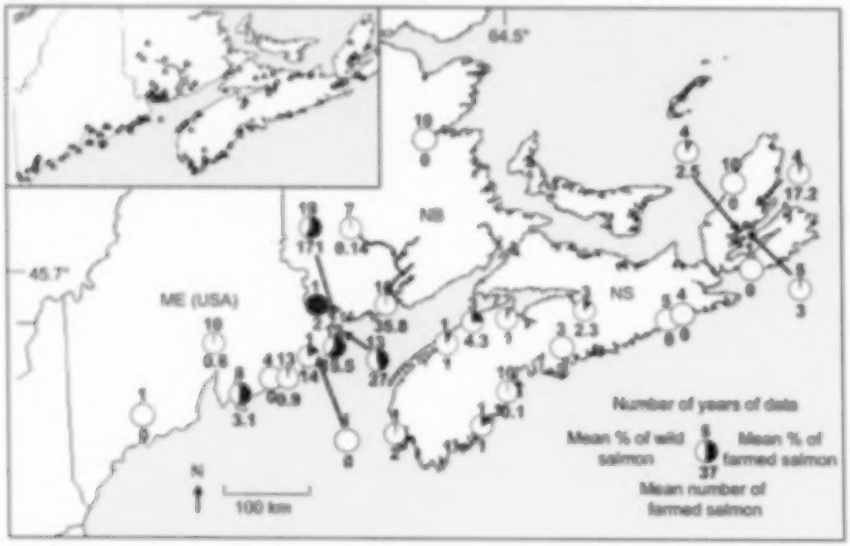

Table 3. Regional summary of information on the prevalence of escaped farmed Atlantic salmon (*Salmo salar*) in investigated eastern North American rivers.

Region	Mean % of adult farmed salmon	No. of rivers with adult farmed salmon	No. of rivers with farmed smolts	No. of rivers with farmed parr	Total no. of rivers with farmed salmon	Detection rate (no. of successful searches/total)
ME	20.2	11 of 13	1 of 1	9 of 9	16 of 17	26/61
NB	16.8	13 of 14	2 of 2	10 of 13	20 of 21	53/78
NL	0.4	2 of 2	1 of 1	N/A	2 of 2	8/25
NS	1.8	16 of 21	N/A	N/A	16 of 21	28/71
All	9.2	42 of 50	4 of 4	19 of 22	54 of 62	114/235

SFAs 16 (Miramichi and southwestern Gulf of St. Lawrence, NB), 18 (Northumberland Strait and western Cape Breton, NS), and 20 (eastern shore NS), no farmed salmon were reported. SFA 21 (southern shore NS) had a mean adult farmed salmon proportion of 0.9% (range 0% to 17%).

The highest number of farmed salmon counted in a river in a single year occurred in NB's Magaguadavic River in which nearly 1200 farmed salmon were captured in the fishway in 1994. Although the numbers have decreased dramatically since then, the proportion has remained relatively stable because of declining wild stocks (Fig. 2). The highest counts of farmed salmon detected in ME occurred in the Dennys River in 2001 (65 found) and 1994 (42 found). In NS, the highest counts of farmed salmon were 55 in the North River (1998) and 23 in the Baddeck River (1995), both of which are located in Cape Breton. In 1997, 10 farmed salmon were reported in NL's Conne River, the highest recorded count for a river in that province. Given that this count was based on a subset of a sample, the true number of farmed salmon was almost certainly higher.

Discussion

Our review supports the contention that escaped farmed salmon are prevalent in rivers of eastern NA. Specifically, escaped farmed salmon have been reported in 54 rivers and bays in the region, or 87% of the watersheds that have been investigated since the inception of the salmon aquaculture industry in 1980. Most of the investigated rivers are located in the heart of the salmon aquaculture industry (Quoddy region, ME and NB), so this percentage would likely be lower if more wild salmon rivers outside of the aquaculture region were investigated. Nevertheless, escaped farmed salmon have been found continuously in appreciable numbers in the region extending from ME to Cape Breton, and this includes some rivers that are not in the primary aquaculture region

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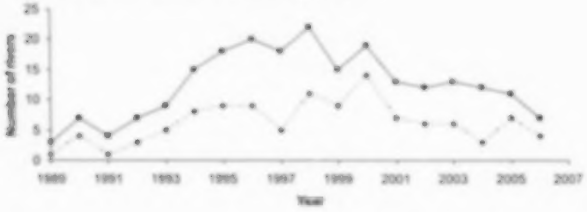
Fig. 6. Effort used in detecting farmed Atlantic salmon (*Salmo salar*) on an annual basis versus success in finding farmed salmon. The solid line represents the total number of investigated rivers in any given year; the broken line represents the number of rivers in which farmed salmon were actually found.



(e.g., Stewiacke River, NS; North River, Cape Breton, NS; Nictaux River, NS). Furthermore, the overall mean proportion of adult farmed salmon relative to wild salmon in investigated rivers over investigated years in eastern NA is estimated to be 9.2%. The proportion of farmed salmon entering rivers with annual adult runs of wild salmon has also been high (>20%) in at least one year in nearly half of the investigated rivers for which proportions were known. These proportions have fluctuated temporally, possibly peaking during mass escape events. The presence of farmed salmon in a river is often not limited to one event; that is, 80% of investigated rivers contained adult farmed salmon in multiple years. Finally, the number of rivers in which farmed salmon are detected in any given year closely reflects the search effort allocated. Collectively, the spatially broad occurrence of escaped farmed salmon in rivers of eastern NA should be cause for concern, given the conservation status of many wild salmon populations in the region (World Wildlife Fund (WWF) 2001; COSEWIC 2006) and the potentially negative impacts of such intrusions on the persistence of wild salmon (Hindar et al. 1991; Weir and Grant 2005; Hutchings and Fraser 2008).

Escape events

In eastern NA, the number of recorded escapes of farmed salmon from sea cages and freshwater hatcheries that we documented (n = 27) is most certainly an underestimate. Since 2003, aquaculture facilities in ME have been required to report escapes of 50 or more 2+ kg salmon; currently, there are no similar government regulations in eastern Canada (Naylor et al. 2005). Interestingly, reported escape events in our review coincided with peaks in annual mean farmed salmon proportions for most years. It is, therefore, possible that unexplained peaks in the numbers of farmed salmon entering rivers result from unreported escapes. The presence of sea cage farmed salmon in rivers in other, "non-peak" years could also be attributed to returning farmed salmon that were able to survive the winter at sea, or to the unreported "leaking" of farmed salmon from sea cages and hatcheries (Stokesbury et al. 2001; Naylor et al. 2005). In the context of managing the risk posed to wild salmon, these patterns, albeit speculative, suggest a means to reduce farmed–wild salmon interactions. With regards to catastrophic, large-scale escapes, "clean-ups" could be planned for nearby rivers to remove as many of these fish as possible during the next spawning run (e.g., Porter 2005). The planning and implementation of farm operation procedures with the aim of reducing such escapes could reduce or possibly eliminate "trickle losses". In Maine, growers have implemented a Hazard Assessment Critical Control Point process (HAACP) to specifically address this issue for both sea cage sites and freshwater hatcheries (Goode and Whoriskey 2003). The public reporting of escape event data would help mobilize the resources to mitigate escape events, engage a broader audience in thinking about solutions for problems, and bolster public confidence in the sustainability of the industry and wild fisheries. For these practical reasons, it is highly advisable that Canadian aquaculture facilities be required to report escapes to governmental agencies with that information then being made accessible to the public.

Incidence of farmed salmon in eastern North America and comparisons with Europe

Our documentation of 54 rivers containing escaped farmed salmon is significantly higher than 14 rivers reported by Ritter et al. (1999) and the estimate of 25 rivers documented by the North American Commission of NASCO (O'Neil et al. 2005). Our literature search found past records that had been missed by previous authors and new records from field teams investigating new sites. Nonetheless, our estimate of 54 rivers should be considered an underestimate, given that some rivers near aquaculture facilities have never been investigated or have been inadequately searched either spatially or temporally. Of the 15 rivers for which there were five years or more of data, we found that 80% contained escaped farmed salmon in multiple years, but not in every year.

The overall mean proportion of adult farmed salmon, across all years, for all investigated eastern NA rivers, was 9.2% of the wild spawning run, with a yearly range from 1990–2006 of 0.2% to 36.3%. This range is similar to that reported for rivers in Norway (yearly mean from 1989–2000: 11%–35%; Fiske and Lund 1999; Fiske et al. 2001, 2006). Alternatively, the United Kingdom, which produces approximately three times more farmed salmon than eastern NA (primarily on the west coast of Scotland), has reported the incidence of farmed salmon in the spawning runs of wild populations to be only 0.5% in Northern Ireland, 4.4% in Scotland (Walker et al. 2006), and 3.2% in England (Milner and Evans 2003). Escapees from these regions may be entrained by prevailing ocean currents (e.g., Whoriskey et al. 2006) and moved north to the coast of Norway (Hansen 2006). If this is true, available data in Europe may underestimate escapes in the UK and overestimate those in Norway.

Fate of escaped farmed salmon

Although the numbers of farmed salmon in rivers often peaked after large-scale escape events, the percentages of escaped fish that have been recovered has always been low and seems to be decreasing with time. For example, in 2000, approximately 128 000 adult salmon escaped from ME and NB aquaculture sites; in 2000–2001, 0.3% (n = 367) of the total number of escapees were recovered in rivers. A similar pattern was noted for 2005 and 2006 (0.5%). In contrast, after an escape event of 20 000–40 000 salmon in 1994, 5.5%–11% (n = 2200) of the farmed salmon were recovered

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in ME and NB rivers in 1994–1995, despite lower search effort than in 2005–2006. There has been a general trend towards lowered farmed salmon numbers in the St. Croix, Saint John, and Dennys rivers as well.

The apparent decrease in the recovery of escaped salmon in rivers could be attributable to several factors. First, it is likely that more escapes occurred in 1994 than were reported; however, to achieve the same recapture rate as 2000–2001 and 2005–2006, 730 000 salmon would have had to have escaped, a number considerably higher than that of any other year. A second possibility is that farmed salmon are experiencing the same high mortalities at sea that are currently threatening wild salmon populations (ASF 2006). This could be evidenced by the relative stability of the proportion of farmed salmon in the Magaguadavic River despite decreasing wild stocks and large escape events. Third, farmed salmon may be less equipped to cope with the natural environment now than they were 10 years ago due to successive generations of domestic breeding (Fleming et al. 2000; Roberge et al. 2006). Fourth, predation rates of farmed salmon could have increased if predators have learned to target the escaped fish (e.g., Whoriskey et al. 2006). Fifth, the 1994 escape event occurred immediately prior to the spawning period, increasing the probability that farmed salmon would return to the rivers and again highlighting the need for aquaculture companies to report escape events immediately. Sixth, in 1994, >50% of the salmon aquaculture industry's smolts were raised in the Magaguadavic watershed, potentially imprinting the farmed smolts to the Magaguadavic and increasing their likelihood to home in to it (e.g., Whoriskey and Carr 2001). Whatever the causes, more studies such as that undertaken by Whoriskey et al. (2006) are needed to establish the fate of farmed salmon at sea.

Potential caveats and data deficiencies

Although the numbers and proportions of farmed salmon may be somewhat biased, any potential biases could not be accounted for in our calculations. Biases could be caused by, for example, the time period during which the salmon run counts were made. Those counts that persisted over a longer time have a greater chance of being accurate, as the numbers of farmed salmon returning to rivers can vary considerably from month to month (Carr et al. 1997; Fiske et al. 2006). The counting method can also affect the proportions and numbers of farmed salmon that are recorded (mark–recapture studies are estimates, swim and shore counts only visually identify salmon, weirs do not capture every salmon going upriver). The method used to determine the origins of the salmon could also bias farmed salmon counts, as identification based on external characteristics (fin erosion, body size, etc.) has led to an underestimation of adult farmed salmon in the past (e.g., Union River, ME, in 2001), and before 2004, there was not a reliable way to identify farmed salmon adults that had escaped as juveniles (Lacroix and Stokesbury 2004). Also, there is the problem of misclassifying a hatchery-released salmon as a farmed escapee, and vice versa. Scale analyses and genetic testing are the most reliable means of identifying farmed salmon but have not been employed in every investigated river. All of these potential biases highlight the need for a standardized way of investigating rivers for the presence or absence of farmed salmon.

Our review also identifies gaps in the spatial distribution of data and thus areas in which further investigation for farmed salmon in eastern NA is merited. Effort was highest in existing major areas of salmon farming in NB, ME, and Cape Breton (NS), but was low along the South Shore of Nova Scotia. This is despite the fact that Nova Scotia's salmon aquaculture industry has experienced a 900% increase in production from 1995 to 2005 (Statistics Canada 2006), with the majority of salmon farming activity occurring along the South Shore. Effort was also low in areas located considerable distances from salmon farming and was almost entirely absent east of the Saint John River, NB. Overall, search effort peaked in 1998 but has been steadily decreasing ever since, despite an increase in farmed salmon production in eastern Canada and several mass episodic escapes.

On a temporal scale, ME, NB, and NL have the most complete data set for certain rivers, having continuous counts for approximately one decade beginning in the mid-1990s for nine rivers (Dennys, Narraguagus, Penobscot, and Union rivers in ME; Magaguadavic, Saint John, and St. Croix rivers in NB; Conne and Little rivers in NL). These long-term ecological data sets are vital to determining the potential impact of farmed–wild salmon interactions (Hutchings 1991; Hindar et al. 2006). Although there are no continuous data sets for any river in NS, despite the presence of farmed salmon in more rivers than ME, NB, or NL, the effort in NS has, in terms of numbers of rivers searched, been greater than that expended in any other region.

There is also a bias in the data with respect to age class. Almost all the reported data are based on adult counts; very little effort has been directed towards the detection of farmed parr and smolts (but see Stokesbury and Lacroix 1997; Stokesbury et al. 2001; Carr and Whoriskey 2006). This relative lack of effort is unfortunate given the observation that farmed juvenile escapees in Europe have a tendency to return to their river of origin as adults (Hansen and Jonsson 1991), whereas adult escapees tend to disperse widely (Hansen 2006), and given that juvenile escapees appear to be more reproductively successful as adults than adult escapees (Fleming et al. 1997). Although episodic escapes of farmed parr and smolts may not be frequent, mass escapes can still occur, e.g., the escape of 100 000 farmed parr during a storm in NL in 1995 (Fisheries and Oceans Canada 1996). Until 2004, standard scale analyses were unable to identify farmed salmon adults that had escaped as juveniles (Lacroix and Stokesbury 2004); thus there has been ample time for farmed–wild introgression to be initiated in wild salmon populations. A comprehensive evaluation of the true impact of such introgression in eastern NA is difficult to undertake given that so little data have been collected on the incidence of farmed salmon in rivers and the effects of farmed–wild interbreeding on wild salmon fitness.

It should also be noted that although this paper focuses exclusively on Atlantic salmon, there are other nonindigenous salmonid species that are being farmed and are escaping in eastern NA. Escaped farmed rainbow trout (*Oncorhynchus mykiss*) have been recorded in 35 NL rivers alone (O'Neil et al. 2005), in addition to several other rivers in NB and NS.

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Implications for risk assessment

Our review provides a comprehensive first step in assessing the scale and scope of the impact that escaped farmed salmon may have on wild salmon populations in eastern NA. Limited work has been done on farmed–wild salmon interactions, with most of it being done in Europe (e.g., Fleming et al. 1997; McGinnity et al. 1997, 2003). Results suggest that farmed salmon can exhibit lower genetic variability than wild salmon (Norris et al. 1999) and that the introgression of farmed salmon genes into a wild population can be comparatively rapid (Garant et al. 2003; Weir et al. 2004). This combination can result in a form of genetic "homogenization" (Fleming et al. 2000), such that the limited genetic variability of the farmed salmon accounts for an increasing proportion of the total population variability (Tufto and Hindar 2003). The consequences of this genetic introgression on fitness, in addition to ecological factors such as competition and disease, are hypothesized to be, in general, negative (reviewed in Jonsson and Jonsson 2006; Hutchings and Fraser 2008; Thorstad et al. 2008). Despite this body of research, little has been done to date to determine whether the consequences of farmed salmon to wild salmon fitness in eastern NA are likely to be similar to those documented in Europe.

What is known in eastern NA is that farmed salmon are appearing in rivers at an overall mean of 9.2% of the spawning run, but occurring in some rivers in some years at proportions exceeding 80%. It is also known that large episodic escapes of farmed salmon occur and that farmed salmon are often found in the same river over multiple years. Furthermore, the successful spawning of farmed salmon has been recorded in several eastern NA rivers (Carr et al. 1997; Lage and Kornfield 2006). Roberge et al. (2006) showed that the farmed salmon strain originating from the Saint John River (NB) exhibited heritable changes in gene expression after five to seven generations of domestication. Most recently, Fraser et al. (2008) showed that crossing Saint John River farmed salmon with wild salmon from one NS population resulted in outbreeding depression in first-generation (F1) farmed–wild hybrid progeny under wild-like conditions. Complexities in the fitness rebound of later generation farmed–wild hybrids (e.g., F2, backcrosses) suggested that divergent mechanisms affect the performance of different farmed–wild hybrids (Fraser et al. 2008). Thus, although limited, available data in eastern NA suggest that the potential risk of both genetic homogenization and a loss of local adaptation in NA wild Atlantic salmon populations due to introgression with farmed fish should be considered high.

Given the implications of our findings from a risk-assessment perspective, we offer the following recommendations (see also Hansen and Windsor 2006). First, escapes of farmed salmon must be immediately reported by aquaculture companies to the government in regions where this is not already done, and this information should be made available to the public. This is an essential step in that those rivers in which the escaped farmed salmon are likely to appear can be monitored and the escapees recovered before they have the opportunity to spawn. This information will provide an empirical basis for the parameterization of ecological impact models. The report should include the numbers of salmon that escaped, the date and time of the escape, the size and life stage (including maturity status) of the salmon, and their health status (Hansen and Windsor 2006). Second, farmed salmon should be marked in such a way that they can be traced back to the aquaculture site of origin, and initiatives to favor continued improvements in containment at farms should be initiated. These initiatives could include improvements in sea cage technologies and sites and in the formation of a clear regulatory environment. Third, methods need to be developed and implemented to identify commercially produced juvenile salmon that escape to fresh water and the offspring of farmed or farmed–wild hybrids in fresh water. Methods are also needed to distinguish farm-origin juveniles from endangered juvenile salmon that have been reared for varying periods in hatcheries before being released to the wild as part of restoration efforts. Identification methods need to be standardized and consistently used in all investigated rivers.

The declining abundance of wild Atlantic salmon, coupled with the ever-increasing production of salmon of aquaculture origin, draws attention to the fundamental need for empirically rigorous assessments of potential risks associated with interactions between wild and farmed individuals. Our compilation of all existing data on escaped farmed salmon in eastern NA is intended to contribute to such a process.

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Don P. Rothaus, Brent Vadopalas, and Carolyn S. Friedman	**2703–2711**	Precipitous declines in pinto abalone (*Haliotis kamtschatkana kamtschatkana*) abundance in the San Juan Archipelago, Washington, USA, despite statewide fishery closure
M.H.H. Price, C.T. Darimont, N.F. Temple, and S.M. MacDuffee	**2712–2718**	Ghost runs: management and status assessment of Pacific salmon (*Oncorhynchus* spp.) returning to British Columbia's central and north coasts
Marie-Elodie Perga, Martin Kainz, and Asit Mazumder	**2719–2727**	Terrestrial carbon contribution to lake food webs: could the classical stable isotope approach be misleading?
Margit Eero, Friedrich W. Köster, and Brian R. MacKenzie	**2728–2741**	Reconstructing historical stock development of Atlantic cod (*Gadus morhua*) in the eastern Baltic Sea before the beginning of intensive exploitation
S.B. Munch and L.M. Clarke	**2742–2751**	A Bayesian approach to identifying mixtures from otolith chemistry data
Steven T. Kalinowski, Michael H. Meeuwig, Shawn R. Narum, and Mark L. Taper	**2752–2760**	Stream trees: a statistical method for mapping genetic differences between populations of freshwater organisms to the sections of streams that connect them
Tracy Noel Rogers and David R. Chalcraft	**2761–2768**	Pond hydroperiod alters the effect of densitydependent processes on larval anurans
Lars G. Rudstam, Frank R. Knudsen, Helge Balk, Gideon Gal, Brent T. Boscarino, and Thomas Axenrot	**2769–2779**	Acoustic characterization of Mysis relicta at multiple frequencies
Miriam Blank, Karl Jürss, and Ralf Bastrop	**2780–2790**	A mitochondrial multigene approach contributing to the systematics of the brook and river lampreys and the phylogenetic position of *Eudontomyzon mariae*
Marianne Nilsen, Torstein Pedersen, Einar Magnus Nilssen, and Stein Fredriksen	**2791–2806**	Trophic studies in a high-latitude fjord ecosystem — a comparison of stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) and trophic-level estimates from a mass-balance model
	PERSPECTIVE	PERSPECTIVE
Matthew R.J. Morris, Dylan J. Fraser, Anthony J. Heggelin, Frederick G. Whoriskey, Jonathan W. Carr, Shane F. O'Neil, and Jeffrey A. Hutchings	**2807–2826**	Prevalence and recurrence of escaped farmed Atlantic salmon (Salmo salar) in eastern North American rivers

Author Index for Volume 65	**AI-1**	**Index des auteurs pour le volume 65**	**AI-1**
Contents for Volume 65	**C-1**	**Sommaire pour le volume 65**	**C-1**

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Volume 65, numéro 12, décembre 2008

	ARTICLES	ARTICLES
Sean C. Anderson, Heike K. Lotze, and Nancy L. Shackell	**2553–2571**	Evaluating the knowledge base for expanding lowtrophic-level fisheries in Atlantic Canada
Bryan A. Black, Darlene C. Gillespie, Shayne E. MacLellan, and Claudia M. Hand	**2572–2578**	Establishing highly accurate production-age data using the tree-ring technique of crossdating: a case study for Pacific geoduck (*Panopea abrupta*)
Jean-Denis Dutil, Coraline Jabouin, Richard Larocque, Gaston Desrosiers, and Pierre U. Blier	**2579–2591**	Atlantic cod (*Gadus morhua*) from cold and warm environments differ in their maximum growth capacity at low temperatures
Matthew L. Keefer, Robert H. Wertheimer, Allen F. Evans, Charles T. Boggs, and Christopher A. Peery	**2592–2605**	Iteroparity in Columbia River summer-run steelhead (*Oncorhynchus mykiss*): implications for conservation
William S.C. Gurney, Philip J. Bacon, Graham Tyldesley, and Alan F. Youngson	**2606–2622**	Process-based modelling of decadal trends in growth, survival, and smolting of wild salmon (*Salmo salar*) parr in a Scottish upland stream
Benjamin D. Walther and Simon R. Thorrold	**2623–2635**	Continental-scale variation in otolith geochemistry of juvenile American shad (*Alosa sapidissima*)
Philipp Emanuel Hirsch and Philipp Fischer	**2636–2643**	Interactions between native juvenile burbot (*Lota lota*) and the invasive spinycheek crayfish (*Orconectes limosus*) in a large European lake
Amy M. Kamarainen, Freya E. Rowland, Reinette Biggs, and Stephen R. Carpenter	**2644–2655**	Zooplankton and the total phosphorus – chlorophyll a relationship: hierarchical Bayesian analysis of measurement error
Mark S. Poos, Nicholas E. Mandrak, and Robert L. McLaughlin	**2656–2666**	A practical framework for selecting among single-species, community-, and ecosystem-based recovery plans
S. Ma eika P. Sullivan and Mary C. Watzin	**2667–2677**	Relating stream physical habitat condition and concordance of biotic productivity across multiple taxa
Meri Härmä, Antti Lappalainen, and Lauri Urho	**2678–2688**	Reproduction areas of roach (*Rutilus rutilus*) in the northern Baltic Sea: potential effects of climate change
Malorie Gélinas and Bernadette Pinel-Alloul	**2689–2702**	Relating crustacean zooplankton community structure to residential development and land-cover disturbance near Canadian Shield lakes

Continued on inside back cover / Suite au verso

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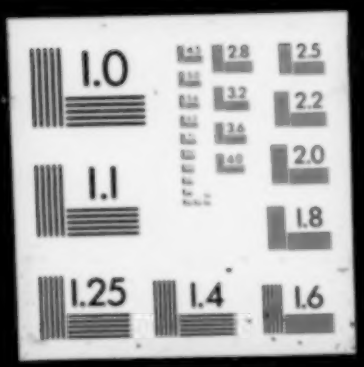
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